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Seasonal dynamics behind the CO_2 efflux of adult European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*) trees – uncovered by stable C isotopes.

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Results of this doctoral thesis have partly been published in or submitted for peer-review to international journals. The following publications are included as individual chapters:

Chapter 2: **Kuptz D**, Matyssek R, Grams TEE (2011) "Seasonal dynamics in the stable carbon isotope composition ($\delta^{13}C$) from non-leafy branch, trunk and coarse root CO_2 efflux of adult deciduous (*Fagus sylvatica*) and evergreen (*Picea abies*) trees", *Plant, Cell & Environment*, 34 (3): 363-373

Chapter 3: **Kuptz D**, Fleischmann F, Matyssek R, Grams TEE (2011) "Seasonal patterns of carbon allocation to respiratory pools in 60-year-old deciduous (*Fagus sylvatica*) and evergreen (*Picea abies*) trees assessed via whole-tree stable carbon isotope labeling", *New Phytologist*, submitted for publication

In addition, the following publication is added as Appendix A and referred to within the text:

Appendix A: Grams TEE, Werner H, **Kuptz D**, Ritter W, Fleischmann F, Andersen CP, Matyssek R (2011) "A free-air system for long-term stable carbon isotope labeling of adult forest trees", *Trees – Structure and Function*, DOI: 10.1007/s00468-010-0497-7

The candidate's individual contribution to each publication is outlined at the end of this thesis.

Summary

The present study examined seasonal pattern of non-leafy branch, trunk and coarse root CO_2 efflux from adult European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* Karst.) trees. In the first part, particular interest was paid on seasonal dynamics in the naturally occurring stable carbon (C) isotope composition ($\delta^{13}C$) of woody tissue CO_2 efflux ($\delta^{13}C_E$), whereby changes in natural $\delta^{13}C_E$ may provide valuable information on the metabolic activity of respiratory pathways. In the second part, the contribution of recent photosynthates to woody tissue respiration and its associated respiratory C pools were quantified at different times during the year. Therefore, recent photosynthates of whole-tree canopies were labeled with stable C isotopes. In particular, labeling was used to elucidate whether the growth respiratory flux from woody tissue is exclusively supplied by recent photosynthates or also by older C. In addition, in both parts of this study, the impact of xylem dissolved respiratory CO_2 on woody tissue $\delta^{13}C_E$ was examined. The present study was founded by the DFG (German Research Foundation) as part of the interdisciplinary research program "SFB 607 – Growth and parasite defense" – project B5.

All experiments were conducted on adult individuals of beech and spruce trees (n = 7, each) at the forest research site "Kranzberger Forst" from May 2008 through June 2009. CO_2 efflux rate of non-leafy branches, upper trunks, lower trunks and coarse roots was measured by a flow-through gas-exchange chamber system. Plexiglass® chambers were attached to individual tree segments and darkened with aluminized polyester foil. A known stable reference gas was flushed through chambers at constant rate. CO_2 efflux rate derived from the offset between reference and chamber air, assessed through infra-red gas analysis. Growth and maintenance respiration were modeled by seasonal changes in the relation between bark temperature and CO_2 efflux rate. Chamber and reference air was collected by an automated gas sampler for the assessment of $\delta^{13}C$ by isotope ratio mass spectrometry and $\delta^{13}C_E$ was calculated using a two-end-member mixing model. Seasonal assessment of $\delta^{13}C_E$ was conducted once per month and species.

Above-ground organs of both species displayed strong and similar seasonal dynamics in their natural occurring $\delta^{13}C_E$. During the main vegetation period, $\delta^{13}C_E$ of non-leafy branch and trunk CO_2 efflux was rather stable (beech: - 28.2 ± 0.2 ‰, spruce: -26.8 ± 0.2 ‰), whereas $\delta^{13}C_E$ continuously

increased during winter dormancy by 5.6 - 9.1 ‰. Seasonal dynamics in $\delta^{13}C_E$ of above ground organs were assumed to be related to the switch from lignin to starch synthesis towards leaf senescence, whereas the continuous increase during winter may be explained by remobilization of starch, temperature sensitivity of the TCA cycle and accumulation of malate by phospho*enol*pyruvate carboxylase (PEPc). Winter photosynthesis most likely damped the seasonal pattern in branches of beech and upper trunks of spruce. In contrast to above-ground organs, seasonal dynamics in $\delta^{13}C_E$ of coarse roots were less pronounced. Low variation in coarse root $\delta^{13}C_E$ was assumed to be related to high pentose phosphate pathway (PPP) activity. The $\delta^{13}C_E$ of spruce was always ^{13}C enriched compared to beech. This offset was related to overall lower ci/ca ratios in conifer needles.

Stable C isotope labeling of recent photosynthates was accomplished by the exposure of ^{13}C depleted CO_2 into tree canopies ($\delta^{13}C \approx -46$ ‰). A free-air CO_2 exposure system ("isoFACE") was used for the continuous release of CO_2 into canopies for five consecutive days. The rate of C translocation and the fraction of labeled C in CO_2 efflux from trunks and coarse roots were quantified for at least nine days. Three experiments were done per species at different phenological stages, i.e. during spring at the end of leaf development, during early summer at maximum growth respiration rates and during late summer at declining growth respiration. In each species, four trees were labeled. Three control trees were monitored simultaneously.

Labeling experiments revealed distinct seasonal pattern in allocation of recently synthesized photosynthates in beech and spruce. Overall, C translocation rates (beech: 0.15 – 0.72 m h^{-1}, spruce: 0.06 – 0.44 m h^{-1}) were highest during early summer compared to spring and late summer, and were interpreted to result from high C sink strength of growth respiration. Phloem sap velocity of beech was about twice as high as compared to that of spruce most likely due to anatomical differences in woody tissue, e.g. the small sieve cells in conifer trees. The fraction of labeled C in CO_2 efflux of beech was low during spring and highest during early summer (coarse roots: 40% of CO_2 efflux from labeled C) and late summer (trunks: 60% of CO_2 efflux from labeled C). Results of beech were related to a two C pool model with one transfer and one storage pool. During spring, growth and maintenance respiration of beech were mainly supplied by storage C. During early summer, recent photosynthates were used to supply growth respiration, whereas during late summer, label was mainly incorporated into the storage pool. Labeling of deciduous beech revealed high dependency on build-up and consumption of C stores. In contrast, evergreen spruce did not display seasonal changes in label abundance within

the CO_2 efflux. The fraction of label in spruce CO_2 efflux was always below 25% indicating that recent photosynthates constantly mixed with older respiratory substrate. A clear distinction of a transfer and a storage pool, as observed for beech, was less pronounced for the evergreen species. Overall, the hypothesis that growth respiration was exclusively supplied by recent photosynthates could not be confirmed in either species. However, it may be valid for beech during early summer.

The above named findings of the first and the second part are consistent in that woody tissue CO_2 efflux of beech and spruce was determined mainly by autotrophic respiration, and that xylem dissolved respiratory CO_2 had only marginal effects on both CO_2 efflux rate and its $\delta^{13}C_E$.

The combination of both the "natural $\delta^{13}C_E$" (part 1) and "labeling" (part 2) approach revealed a coherent picture on seasonal dynamics of beech and spruce CO_2 efflux. Overall, tree phenology largely affected woody tissue CO_2 efflux, apparently due to changing metabolic activity of respiratory pathways and changes in the contribution of different respiratory substrate pools. Seasonal dynamics by tree phenology were always more pronounced in the deciduous than in the evergreen species. The present study highlighted the fact that the influence of tree phenology has to be considered to fully understand woody tissue CO_2 efflux. Thereby, phenology effects have to be taken into account by investigating forest ecosystem respiration.

Zusammenfassung

Die vorliegende Dissertation untersuchte saisonale Muster im CO_2-Efflux unbelaubter Zweige, Stämme und Grobwurzeln adulter Rotbuchen (*Fagus sylvatica* L.) und Fichten (*Picea abies* Karst.). Der erste Teil der Arbeit befasste sich dabei mit saisonalen Änderungen in der natürlichen Kohlenstoff-Isotopenzusammensetzung ($\delta^{13}C$) des CO_2-Effluxes ($\delta^{13}C_E$), wobei die Änderung im natürlichen $\delta^{13}C_E$ wertvolle Informationen über die metabolische Aktivität einzelner Atmungswege geben kann. Im zweiten Teil der Arbeit wurde der Anteil neu gebildeter Assimilate an der Holzatmung und den ihr zugeordneten Kohlenstoffpools zu unterschiedlichen Zeitpunkten im Jahr quantifiziert. Hierzu wurden neu gebildete Photosyntheseprodukte kompletter Baumkronen mit stabilen Kohlenstoffisotopen markiert. Insbesondere wurde auf die Frage eingegangen, ob Wuchsatmungsprozesse ausschließlich aus neuen Photosyntheseprodukten oder aber auch aus älterem Kohlenstoff gespeist werden. In beiden Teilen der Arbeit wurde zudem die Frage untersucht, ob im Xylemfluss gelöstes CO_2 einen Einfluss auf den $\delta^{13}C_E$ des Gewebes hat. Die vorliegende Studie war Teil des von der Deutschen Forschergemeinschaft (DFG) finanzierten interdisziplinären Sonderforschungsbereichs „SFB 607 – Wuchs und Parasitenabwehr - Wettbewerb um Ressourcen in Nutzpflanzen aus Land- und Forstwirtschaft“ – Projekt B5.

Alle Experimente wurden auf der Versuchsfläche „Kranzberger Forst“ an adulten Buchen und Fichten von Mai 2008 bis Juni 2009 durchgeführt (Individuen pro Baumart: $n = 7$). Die CO_2-Effluxrate unbelaubter Zweige, oberer und unterer Stämme, sowie von Grobwurzeln wurde mit Hilfe eines offenen Gaswechsel-Kammer-Systems erfasst. Plexiglass® - Kammern wurden an Zweigen, Stämmen und Grobwurzeln angebracht und mit aluminisierter Polyesterfolie abgedunkelt. Die Kammern wurden mit einem stabilen Referenzgas bei konstanter Geschwindigkeit gespült. Die CO_2-Effluxrate wurde als Unterschied zwischen Kammer- und Referenzluft mittels Infrarot-Gasanalyse bestimmt. Wuchs- und Unterhaltsatmung wurden aus saisonalen Dynamiken der Rindentemperatur und der CO_2-Effluxrate modelliert. Gasproben von Kammer- und Referenzluft wurden mit Hilfe eines automatisch Gas-Sampler gesammelt. Der $\delta^{13}C$ dieser Gasproben wurde mittels eines Isotopen-Ratio-Massenspektrometers bestimmt und zur Berechung des $\delta^{13}C_E$ verwendet. Messungen zur saisonalen Änderungen im natürlichen $\delta^{13}C_E$ wurde einmal pro Monat und Baumart durchgeführt.

Die oberirdischen Organe beider Baumarten zeigten ausgeprägte und sich einander ähnelnde saisonale Dynamiken im natürlichen $\delta^{13}C_E$. Der $\delta^{13}C_E$ unbelaubter Äste und Stämme war während der Hauptvegetationsperiode relativ konstant (Buche: -28.2 ± 0.2 ‰, Fichte: -26.8 ± 0.2 ‰), wohingegen er während der Winterruhe um 5.9 bis 9.1 ‰ kontinuierlich anstieg. Als Ursache für saisonale Schwankungen im $\delta^{13}C_E$ der oberirdischen Organe wurden zum einen der Übergang von Lignin zu Stärkesynthese zu Beginn der Blattseneszenz, zum anderen Remobilisierung von Stärke, die Temperatursensitivität des TCA Zyklus und Akkumulation von Malat durch das Enzym Phospho*enol*pyruvat Carboxylase im Winter diskutiert. Aktive Photosynthese im Winter schwächte die saisonalen Schwankungen im $\delta^{13}C_E$ sowohl in Zweigen der Buche als auch in den oberen Fichtenstämmen ab. Im Gegensatz zu den oberirdischen Organen waren die saisonalen Dynamiken im $\delta^{13}C_E$ der Grobwurzeln deutlich geringer ausgeprägt. Als Ursache wurde hohe Pentose-Phosphat-Weg-Aktivität (PPP) erörtert. Im direkten Artvergleich war der $\delta^{13}C_E$ der Fichte immer ^{13}C-angereichert im Vergleich zur Buche. Dies wurde durch generell geringere ci/ca Werte in Koniferennadeln erklärt.

Die Markierung neuer Photosyntheseprodukte mittels stabiler Kohlenstoffisotopen geschah durch die Freisetzung von ^{13}C-abgereichertem CO_2 ($\delta^{13}C \approx -46$ ‰) in den Kronenraum kompletter Baumkronen. CO_2 wurde mittels eines Freiluft-CO_2-Begasungssystems („isoFACE“) fünf Tage lang kontinuierlich in die Kronen geleitet. Sowohl die Kohlenstofftransportgeschwindigkeit in den Bäumen als auch der Anteil des markierten Kohlenstoffes am CO_2-Efflux von Stämmen und Grobwurzeln wurden mindestens neun Tage lang nach Begasungsbeginn quantifiziert. Drei Markierungsexperimente wurden pro Baumart durchgeführt. Das erste jeweils im Frühling zum Ende des Blattaustriebes, das zweite im Frühsommer unter maximalen Wuchsatmungsraten und das dritte im Spätsommer, als die Wuchsatmungsrate wieder abnahm. Vier Bäume pro Art wurden markiert, drei weitere dienten als Kontrolle.

Die Markierung neuer Assimilate mit stabilen Kohlenstoffisotopen zeigte ausgeprägte saisonale Muster in der Allokation kürzlich gebildeter Photosyntheseprodukte in Buche und Fichte. Generell war die Kohlenstofftransportrate (Buche: 0.15 – 0.72 m h^{-1}, Fichte: 0.06 – 0.44 m h^{-1}) im Frühsommer bei beiden Baumarten am höchsten und wurde als starker Kohlenstoffbedarf für Wuchsatmungsprozesse gedeutet. Die Phloemtransportgeschwindigkeit der Buche war in der Regel doppelt so hoch wie die der Fichte, was auf anatomische Unterschiede im Holzkörper, z.B. generell kleinere Siebzellen von Koniferen, zurückgeführt wurde. Der Prozentsatz an markiertem Kohlenstoff

im CO_2-Efflux von Buche war im Frühling gering und maximal im Früh- (Grobwurzeln, ca. 40% des CO_2-Effluxes aus markiertem Kohlenstoff), bzw. im Spätsommer (Stämme, ca. 60% des CO_2-Effluxes aus markiertem Kohlenstoff). Die Ergebnisse an Buche wurden mit Hilfe zweier Kohlenstoffpools interpretiert, einem Transfer- und einem Speicherpool. Während des Frühlings wurden sowohl Wuchs- als auch Unterhaltsatmung der Buche aus dem Kohlenstoffspeicher versorgt. Im Frühsommer wurden frische Photosyntheseprodukte für Wuchsatmung, im Spätsommer dagegen für den Aufbau des Speicherpools verwendet. Die Markierungsexperimente an Buche verdeutlichten dabei die hohe Abhängigkeit der wechsellaubigen Art von Speicherauf- bzw. -abbau. Im Gegensatz zur Buche zeigte der Anteil an markiertem Kohlenstoff im CO_2-Efflux von Fichte keine saisonalen Änderungen und überschritt niemals 25%. Die Ergebnisse an Fichte ließen den Schluss zu, dass aktuelle Photosyntheseprodukte immer mit älterem Kohlenstoff durchmischt wurden. Eine deutliche Trennung des Substrats in einen Transfer- und einen Speicherpool war jedoch für die immergrüne Art weniger ausgeprägt. Insgesamt konnte die Hypothese, dass Wuchsatmungsprozesse ausschließlich aus der aktuellen Photosynthese gespeist werden, für keine der beiden Baumarten grundsätzlich bestätigt werden, obwohl sie im Frühsommer für Buche zuzutreffen scheint.

Beide experimentelle Ansätze stimmten darin überein, dass vom Holzkörper abgesondertes CO_2 in adulten Buchen und Fichten hauptsächlich aus autotropher Atmung stammt und dass im Xylemfluss gelöstes Atmungs-CO_2 nur einen geringen Einfluss auf die CO_2-Effluxrate und deren $\delta^{13}C_E$ hat.

Die Untersuchungen zu saisonalen Schwankungen im natürlichen $\delta^{13}C_E$ (Teil 1) in Kombination mit den Markierungsexperimenten (Teil 2) ergab ein in sich stimmiges Bild zu saisonalen Dynamiken im CO_2-Efflux von adulten Buche und Fichte. Die Studie zeigte auf, dass die Phänologie der Bäume einen ausgeprägten Einfluss auf das vom Holzkörper abgesonderte CO_2 hat, offenbar sowohl aufgrund sich saisonal ändernder metabolischer Aktivität einzelner Atmungswege, als auch aufgrund unterschiedlicher Beteiligung einzelner Substratpools. Die durch die Phänologie hervorgerufenen saisonalen Dynamiken waren dabei in der laubwerfenden Art stärker ausgeprägt als in der immergrünen. Insgesamt machte die vorliegende Studie deutlich, dass der Einfluss der Phänologie berücksichtigt werden muss, wenn man die komplexen Zusammenhänge, die den CO_2-Effluxes

adulter Waldbäumen bestimmen, verstehen will. Dies muss insbesondere bei Untersuchungen zur Ökosystematmung von Wäldern beachtet werden.

1. General introduction

Atmospheric carbon dioxide (CO_2) is essential for life on earth, as it contributes to the planet's natural green-house effect (Harries, 1996) and is a main substrate for plant photosynthesis (Von Caemmerer & Farquhar, 1981). The pool of atmospheric carbon (C) is in permanent exchange with terrestrial or maritime ecosystems, called the natural global C cycle (Körner, 2003; IPCC, 2007b). Its main fluxes, i.e. terrestrial photosynthesis and respiration or sea surface gas exchange are nearly balanced (Schimel, 1995) and led to repeated fluctuations in atmospheric CO_2 concentration during the last six glacial periods between 180 and 300 µmol mol^{-1}. In contrast, recent monitoring displayed an almost continuous increase in atmospheric [CO_2] leading up to a 30% higher concentration compared to pre-industrial times (IPCC, 2007b). Scientists world-wide consent that this increase has anthropogenic origin and will inevitably lead to global warming, highlighting the necessity to manage the global C cycle (IPCC, 2007b). However, the general basis for C cycle management is profound understanding of each C flux involved, allowing for correct C cycle modeling as background for fundamental political decisions (Gifford, 2003; Davidson *et al.*, 2006).

Photosynthesis by terrestrial plants represents the largest flux of C out of the atmosphere (about 123 Gt of C $year^{-1}$, IPCC, 2007b) and is nearly balanced by an opposite flux constituting to one half of autotrophic respiration by plants (about 60 Gt of C $year^{-1}$) and to the other half of the sum of heterotrophic soil respiration (including litter decomposition) and fires (about 60 Gt of C $year^{-1}$, Gifford, 2003; IPCC, 2007b). Much of the remaining C is stored into terrestrial biomass, thereby reducing the impact of the anthropogenic [CO_2] increase. Forests, covering nearly 30% of the terrestrial surface area, represent the largest biomass C pool among terrestrial ecosystems (about 500 Gt C (Körner, 2003; FAO, 2009). About 25% of the global forest area is located in Europe (including the Siberian forests), highlighting the importance of these forests within the global C cycle. European forests increase in biomass at an annual rate of 1.07 (FAO, 2006) and are therefore regarded as terrestrial C sinks (Knohl *et al.*, 2003; Luyssaert *et al.*, 2008; Schultze *et al.*, 2010). However, the main determinate whether European forests are C sinks or C sources is ecosystem respiration, and a forest becomes a C source as soon as ecosystem respiration exceeds photosynthetic C fixation (Valentini *et al.*, 2000).

According to Trumbore (2006), we still lack a "theory of respiration", referring to the fact that the term ecosystem respiration integrates over a variety of C fluxes, with large uncertainties on these

fluxes remaining. The largest fraction of ecosystem respiration in European forests is autotrophic respiration, releasing about 44 to 57% of forest gross primary productivity (Ciasis *et al.*, 2010; Luyssaert *et al.*, 2010; Schultze *et al.*, 2010). Autotrophic respiration is often measured directly as the amount of CO_2 emitted by plant tissue (Stockfors & Linder, 1998; Ceschia *et al.*, 2002) or modeled by temperature dependencies (Ryan, 1991; Acosta *et al.*, 2008). However, considering the complexity of plant metabolisms involved, such approaches may be unsuitable to provide comprehensive understanding of autotrophic respiration and may restrict its prognosis under changing environmental conditions (Gifford, 2003; Davidson *et al.*, 2006; Trumbore, 2006).

At the plant physiological level, autotrophic respiration may not be related to a single metabolic process, but integrates over a variety of respiratory pathways. Thereby, the respiratory metabolism releases CO_2 through the step-wise oxidative decarboxylation of organic compounds, mainly carbohydrates but also lipids, amino acids or proteins. Respiration is essential for plant life, as it provides chemical energy for endergonic cell processes in form of adenosine triphosphate (ATP) and reduced nucleotides, such as NADH or NADPH. Furthermore, the respiratory metabolism produces C skeletons for the biosynthesis of organic compounds, e.g. for amino acids or lipids. Main respiratory decarboxylating processes are the dehydrogenation of pyruvate (PDH), the tricarboxylic acid (TCA) cycle and the oxidative stage of the pentose phosphate pathway (PPP, Table 1.1). The sum of CO_2 released by these processes may be considered as the gross respiratory flux. In contrast, the net amount of CO_2 released by plants, i.e. the amount of CO_2 usually measured as autotrophic respiration, is also affected by refixation of respired CO_2 (Table 1.1). In C_3 plants, such carboxylation processes may occur either photosynthethically, i.e. by ribulose-1,5-bisphosphate carboxylase oxygenase (RubisCO) in chloroplasts (Cernusak *et al.*, 2001; Pfanz *et al.*, 2002), or non-photosynthethically, i.e. by phospho*enol*pyruvate carboxylase in the mitochondria (PEPc, Berveiller *et al.*, 2007b; Gessler *et al.*, 2009b). Thus, merely measuring the net amount of CO_2 released by forest trees might capture their net rate of autotrophic respiration, but does not account for the individual contribution of each respiratory flux, which may differ on both spatial and temporal scales (Trumbore, 2006). However, such knowledge is mandatory for reliable modeling of ecosystem C fluxes.

Table 1.1: Decarboxylation and carboxylation processes determining autotrophic respiration in C_3 plants

Process	Denotation	Process description
PDH	Pyruvate dehydrogenase complex	Pyruvate is decarboxylated, whereby its acetyl moiety is transferred to CoA, synthesizing Acetyl-CoA. The latter serves as substrate for TCA and fatty acid synthesis. For each molecule pyruvate, PDH releases one molecule CO_2, synthesizing one molecule NADH.
TCA	Tricarboxylic-acid cycle	Acetyl-CoA is decarboxylated, releasing two molecules CO_2 while producing 24 ATP (mainly by NADH). Furthermore, TCA intermediates such as oxalacetate or α-oxoglutarate are used for amino acid synthesis.
PPP	Penthose-phosphate-pathway	The oxidative stage of the PPP is the major source for NADPH, which is necessary for the synthesis of fatty acids and terpenoids. Furthermore, glucose is reduced to pentoses, serving as substrate for the synthesis of nucleic acid.
RubisCO	Ribulose-1,5-bisphosphate-carboxylase-oxygenase	Photosynthethically fixation of CO_2 during the Calvin cycle to produce triose phosphates for carbohydrate synthesis. Furthermore, RubisCO activity in branches and stems provides additional O_2 for respiratory metabolisms.
PEPc	Phospho*enol*-pyruvate carboxylase	CO_2 is fixed non-photosynthetically as malic acid (via oxalacetate) to supply TCA with citrate intermediates to sustaining TCA activity during Acetyl-CoA shortage caused by lipid synthesis. This supply is termed 'anaplerotic'.

Plant organs such as leaves, trunks, coarse or fine roots execute specific functional tasks, i.e. the production of carbohydrates (leaves) or the uptake of nitrogen (fine roots). Consequently, the activity of each of the above named respiratory pathways may differ among plant organs. For instance, PPP of *Phaesolus vulgaris* (L.) and *Zea mays* (L.) was substantially more active in roots compared to leaves, producing NADPH for the reduction of nitrate in root tissue (Dieuaide-Noubhani *et al.*, 1995; Bathellier *et al.*, 2009). Likewise, *Ricinus communis* (L.) and *Fagus sylvatica* (L.) displayed significantly higher PEPc activity in both roots and trunks compared to leaves, releasing O_2 to prevent anoxia due to O_2 consuming processes (Berveiller & Damesin, 2008; Gessler *et al.*, 2009b). While the activity of respiratory pathways might differ among plant organs, it may also vary on temporal scales, e.g. due to meteorological conditions. In this respect, each respiratory pathway might respond individually. For instance, TCA cycle activity in roots and trunks of *Pinus sylvestris* (L.) and *Quercus petrea* (Liebl.) is assumed to be highly temperature-sensitive and down-regulated at low temperatures (Atkin *et al.*, 2000; Maunoury *et al.*, 2007; Kodama *et al.*, 2008). In contrast, Priault *et al.* (2009) observed substantially up-regulated PDH activity in leaves of *Halimium halimiforium* (L.) at high irradiation. Thus, diurnal changes in environmental conditions may lead to both high and low ratios of

CO_2 released by PDH and the TCA cycle in leaves and woody tissues. In conclusion, changes in meteorological conditions may not only change the contribution of each individual respiratory pathway along temporal scales, but such response might also differ among plant organs.

Metabolic activity of respiratory pathways is affected, in addition, by seasonal changes in growth activity. Plant respiration is often considered as a mixture of both growth and maintenance processes, usually abstracted as growth and maintenance respiration (Stockfors & Linder, 1998; Amthor, 2000). While growth respiration includes all processes providing C skeletons and chemical energy for the biosynthesis of new plant tissue, maintenance respiration integrates processes sustaining the living biomass, i.e. protein turn-over and the replacement of cellular structures. Although the metabolic basis of growth and maintenance respiration is similar, the contribution to autotrophic respiration differs with seasons. Thereby, growth respiration is associated with the construction of new tissue during periods of active plant growth, whereas maintenance respiration depends on environmental conditions, such as temperature (Ryan, 1991). Despite the explanatory usefulness of the growth - maintenance concept (Bowling *et al.*, 2008), the correct separation of autotrophic respiration into growth and maintenance components remains a challenge (Gifford, 2003; Davidson *et al.*, 2006).

Plant phenology not only affects net respiration rates by changing growth activity, but also by determining the type and age of the respiratory substrate. Recent photosynthates are usually transported to sites of high C demand, e.g. to actively growing tissues (Dickson, 1989; Kozlowski, 1992). Allocation of recent photosynthates (Hansen & Beck, 1990; Litton *et al.*, 2007) as well as the build-up and consumption of storage C may change throughout the year (Skomarkova *et al.*, 2006; Maunoury *et al.*, 2007) and up to date, C allocation and the importance of C stores are not well understood. While the contribution of C stores is evident during periods when respiratory loss exceeds photosynthetic C fixation, e.g. in deciduous forests during winter, their contribution to autotrophic respiration during other seasons remains unclear (Trumbore, 2006). In fact, storage build-up and consumption may not only occur at seasonal, but also at diurnal scales, i.e. by accumulation of transitory starch or malate in leaves during the day (Tcherkez *et al.*, 2004; Brandes *et al.*, 2006; Barbour *et al.*, 2007). Large uncertainties remain on the actual amount, identity (i.e. carbohydrates, lipids, etc.) and turnover rates of individual C pools contributing to autotrophic respiration (Carbone & Trumbore, 2007; Lehmeier *et al.*, 2008; Gaudinski *et al.*, 2009). Such information, however, is

necessary to fully understand carbon cycling in forest ecosystems (Trumbore, 2006; Litton *et al.*, 2007).

Finally, CO_2 emitted by plants may not derive from local respiration alone. Recently, large quantities of CO_2 dissolved within the xylem sap have been observed (Teskey & McGuire, 2007; Saveyn *et al.*, 2008). The origin of this CO_2 might be trunk (McGuire & Teskey, 2004), root (Aubrey & Teskey, 2009) and soil respiration (Moore *et al.*, 2008). Teskey *et al.* (2008) argue that the cambium serves as a strong diffusional barrier and that a large quantity of respired CO_2 might not diffuse to the atmosphere but rather get dissolved within the xylem water and transported along the main tree axis. Accordingly, xylem dissolved CO_2 may diffuse to the atmosphere distant from the site of origin, and autotrophic respiration might be under or overestimated by measuring the amount of CO_2 emitted from plant organs (Hölttä & Kolari, 2009). Hence, instead of using the term "autotrophic respiration", the correct term for plant emitted CO_2 is "CO_2 efflux" and will be addressed as such in the present thesis.

To summarize the paragraphs above, the amount of CO_2 emitted by plants, i.e. the CO_2 efflux is a complex network of various individual processes. Large uncertainties remain on spatial and temporal variability of different respiratory metabolic pathways, but also on the size, age, amount and identity of different substrate pools and on the influence of xylem dissolved CO_2. The extent of these knowledge gaps, however, hinders correct predictions about CO_2 efflux rates under changing environmental conditions. Hence, solving such uncertainties is mandatory for reliably predicting ecosystem respiration under future climates. In this respect, the present thesis aimed to broaden our understanding of plant CO_2 efflux by contributing to the resolution of some of the above stated knowledge gaps.

The use of stable C isotopes has proven to be a suitable tool for clarifying C cycling in plants (Dawson *et al.*, 2002; Trumbore, 2006). A variety of plant physiological studies have taken advantage of the fact that natural occurring levels of light (^{12}C) and heavy (^{13}C) stable carbon isotopes, i.e. their "natural abundance", changes during the interaction of plants with their abiotic and biotic environment. Such changes, termed "isotopic fractionation", are mainly caused by the selective enrichment or depletion of one isotope during a chemical or physical process. Reactions causing isotopic fractionation may be distinguished into "kinetic" (i.e. unidirectional, irreversible process with the immediate withdrawal of the product) and "equilibrium" fractionation (i.e. bidirectional process,

reversible), but have in common that one isotope accumulates on one site of the reaction. Thereby the isotopic ratio (R) of the heavier versus the lighter isotope changes between substrate and product. Since the absolute abundance of ^{13}C on the planet is small (about 98.9% ^{12}C and 1.1% ^{13}C, respectively), the mixture of ^{12}C and ^{13}C isotopes is usually presented as the stable C isotope composition ($\delta^{13}C$ in ‰), i.e. the ratio of the heavy to the light isotope in a sample related to the respective ratio of an international standard (Vienna Pee Dee Belemnite Standard, see Dawson *et al.*, 2002):

$$\delta^{13}C = 1000 * \left(\frac{R^{13}C_{Sample}}{R^{13}C_{Standard}} - 1\right) \quad (‰), \qquad \text{Eqn. 1.1}$$

In plants, $\delta^{13}C$ of bulk organic matter (Badeck *et al.*, 2005), soluble organic compounds (Bowling *et al.*, 2008) and respiratory fluxes are different (Tcherkez *et al.*, 2003; Maunoury *et al.*, 2007). Such isotopic imbalances are the result of both photosynthetic and post-photosynthetic fractionation processes (Farquhar *et al.*, 1989; Badeck *et al.*, 2005). In C_3 plants, photosynthetic fractionation is mainly caused by discrimination against ^{13}C during carboxylation by RubisCO and by diffusion through the stomata (Farquhar *et al.*, 1989). Post-photosynthetic fractionation processes include all isotopic effects after the carboxylation by RubisCO (Badeck *et al.*, 2005) and are mainly caused by enzymatic fractionation (Tcherkez & Farquhar, 2005) and by positional isotopic imbalance among C atoms of hexoses (i.e. relative ^{13}C enriched C atoms at positions 3 and 4, Rossmann *et al.*, 1991; Gleixner & Schmidt, 1997). Such positional imbalances lead to a variety of post-photosynthetic fractionation processes during the biosynthesis of organic compounds (Tcherkez *et al.*, 2004), during C allocation (Gessler *et al.*, 2009a) or during autotrophic respiration (Priault *et al.*, 2009; Werner *et al.*, 2009). Thus, understanding the reasons behind changing natural $\delta^{13}C$ values gives valuable information on C utilization of plants.

In addition, stable C isotopes provide the opportunity to directly assess the magnitude and variability of individual C fluxes by labeling of one or more C sources with highly ^{13}C enriched or depleted substances. For instance, such stable C isotope tracers may be applied to tree canopies via atmospheric CO_2 for labeling the pool of recent photosynthates (Högberg *et al.*, 2001; Plain *et al.*, 2009). Also, labeled organic compounds, i.e. positional labeling of pyruvate, may be used to trace individual respiratory pathways (Werner *et al.*, 2009). Such labeling studies help to assess changes in

C allocation (Hansen & Beck, 1990) and to partition individual C fluxes such as the contribution of recent photosynthates to plant and soil respiration (Plain *et al.*, 2009).

In the present thesis, a combination of both the natural abundance and the labeling approaches was used to analyze the autotrophic respiration of adult forest trees. Thereby, the study focused on seasonal dynamics determining the CO_2 efflux of non-leafy plant organs. Measurement campaigns extended throughout a whole year, from May 2008 to June 2009 and were conducted at the forest research site "Kranzberger Forst" as part of the DFG founded research project "SFB 607 – Growth and parasite defense", Project B5. Tree species studied were European beech (*Fagus sylvatica*, L.) and Norway spruce (*Picea abies*, Karst). The results of this study are presented as three individual parts:

The first part deals with seasonal variations in naturally occurring $\delta^{13}C$ of the CO_2 efflux ($\delta^{13}C_E$) of non-leafy branches, trunks and coarse roots of beech and spruce, covering the course of a whole year. To date, only few studies have investigated seasonal changes in $\delta^{13}C_E$ simultaneously of different plant organs and, in particular, the winter period was hardly covered. [The content of this chapter is published in *Plant, Cell & Environment*, 34 (3): 363-373]

The second part describes a series of labeling experiments using the isoFACE labeling infrastructure. [The isoFACE setup is a free-air stable C isotope labeling system. Its description was previously published as Grams et al. (2011) in *Trees – Structure and Function*, DOI: 10.1007/s00468-010-0497-7 and added to this thesis as appendix A]. Labeling was done to assess seasonal changes in the contribution of recent photosynthates to the CO_2 efflux of trunks and coarse roots of beech and spruce. Three C labeling experiments were performed on each tree species at different phenological stages. Main question was whether the growth respiratory flux is supplied exclusively by recent photosynthates or, in addition, by older respiratory substrate. [The content of this chapter is presently submitted for publication in *New Phytologist* (Chapter 3).]

The concluding part (Chapter 4: General discussion) will by a synopsis of chapters 2 and 3, evaluating the experimental setups, highlighting consistencies among the results and elaborating novel evidence resulting from combining the “natural abundance” with the “labeling” approach. A coherent picture on autotrophic respiration of adult beech and spruce trees will be developed, covering changes in metabolic activity, allocation of recent photosynthates and the use of different substrate sources.

2. Seasonal dynamics in the stable carbon isotope composition ($\delta^{13}C$) from non-leafy branch, trunk and coarse root CO_2 efflux of adult deciduous (*Fagus sylvatica*) and evergreen (*Picea abies*) trees[1]

Summary

Respiration is a substantial driver of carbon (C) flux in forest ecosystems and stable C isotopes provide an excellent tool for its investigation. We studied seasonal dynamics in $\delta^{13}C$ of CO_2 efflux ($\delta^{13}C_E$) from non-leafy branches, upper and lower trunks and coarse roots of adult trees, comparing deciduous *Fagus sylvatica* (European beech) with evergreen *Picea abies* (Norway spruce).

In both species, we observed strong and similar seasonal dynamics in the $\delta^{13}C_E$ of above-ground plant components, whereas $\delta^{13}C_E$ of coarse roots was rather stable. During summer growth $\delta^{13}C_E$ of trunks was about -28.2 ‰ (beech) and -26.8 ‰ (spruce). During winter dormancy when growth respiration ceased, $\delta^{13}C_E$ increased by 5.6 - 9.1 ‰. The observed dynamics are likely related to a switch from growth to starch accumulation during fall and remobilization of starch, low TCA cycle activity and accumulation of malate by PEPc during winter. The seasonal $\delta^{13}C_E$ pattern of branches of beech and upper trunks of spruce was less variable, probably because these organs were additionally supplied by winter photosynthesis. In view of our results and pervious studies, we conclude that the pronounced increases in $\delta^{13}C_E$ of trunks during the winter results from interrupted access to recent photosynthates.

[1] Published as Kuptz D, Matyssek R, Grams TEE (2011) to *Plant, Cell & Environment*, 34 (3): 363-373

Introduction

European forests (including Siberia) represent 25% of the world's forest area, covering 44% of the total European land mass (FAO, 2006). Forest ecosystems play a key role within the global carbon (C) cycle, both via C uptake during plant photosynthesis and C loss during ecosystem respiration (Goodale *et al.*, 2002; Nabuurs *et al.*, 2003). The balance between these two processes determines whether European forests act as carbon sinks or sources (Valentini *et al.*, 2000). Plant respiration is a major component of forest ecosystem respiration (Luyssaert *et al.*, 2010) and comprehensive understanding of the complex dynamics behind plant respiratory processes is crucial to predict carbon cycling in forests under future environments (Trumbore, 2006). However, these dynamics are not well understood.

Stable C isotopes are of increasing relevance in the field of carbon cycle research and provide new insights, e.g. into the dynamics of plant metabolic processes (Dawson *et al.*, 2002). Stable C isotopic composition ($\delta^{13}C$ in ‰) is altered by a variety of processes along the C transfer pathway from the atmosphere through the plant to the soil, such as discrimination during gross photosynthesis, tree C allocation, or C loss to the atmosphere via respiration (Bowling *et al.*, 2008). Currently, fractionation by photosynthesis is well understood (Farquhar *et al.*, 1989), but large uncertainties remain concerning post-photosynthetic fractionation processes such as tree-internal C transport or plant respiration (Gessler *et al.*, 2009a). For instance, distinct seasonal changes in the $\delta^{13}C$ of respiratory CO_2 efflux ($\delta^{13}C_E$) from non-leafy branches and trunks of European forest trees have been observed (Damesin & Lelarge, 2003; Maunoury *et al.*, 2007).

Seasonal dynamics in $\delta^{13}C_E$ may originate from the use of different substrates for respiration (Damesin & Lelarge, 2003), i.e. reserves that may differ in their $\delta^{13}C$ compared to recent photosynthates (Bowling *et al.*, 2008). Furthermore, fractionation may occur at metabolic branching points in the respiratory pathway (Ghashghaie *et al.*, 2003; Tcherkez *et al.*, 2003). Glucose molecules have a reproducible, non-statistical ^{13}C distribution with relatively ^{13}C enriched C atoms at the C-3 and C-4 positions (Rossmann *et al.*, 1991). Thus, respiratory processes using C atoms from default positions release either relatively ^{13}C enriched CO_2, e.g. by the pyruvate dehydrogenase (PDH) complex or relatively ^{13}C depleted CO_2, e.g. during the tricarboxylic acid (TCA) cycle (Ghashghaie *et al.*, 2003; Priault *et al.*, 2009; Werner *et al.*, 2009). This "fragmentation fractionation" (Tcherkez *et al.*, 2003) may lead to temporal and spatial variations in $\delta^{13}C_E$, if imbalances between respiratory processes occur. For example, PDH activity is assumed less temperature sensitive than TCA (Atkin *et*

al., 2000). Consequently, its contribution to $\delta^{13}C_E$ would prevail at low temperatures (Kodama *et al.*, 2010), leading to enriched $\delta^{13}C_E$ during winter (Maunoury *et al.*, 2007).

Only few studies have investigated seasonal dynamics on $\delta^{13}C_E$ of adult forest trees (Damesin & Lelarge, 2003, Maunoury *et al.*, 2007), with the winter period hardly covered. Simultaneous measurements on seasonal $\delta^{13}C_E$ of different tree organs, including both above and below-ground components are still lacking (Bowling *et al*., 2008). The present study addresses these shortcomings by investigating the $\delta^{13}C_E$ from 60-year-old forest trees of one evergreen conifer (*Picea abies* Karst.) and one deciduous broad-leaved species (*Fagus sylvatica* L.). Rates of CO_2 efflux and $\delta^{13}C_E$ of upper and lower trunks, coarse roots and branches were compared (the latter only in beech). The most prominent dynamics in $\delta^{13}C_E$ were expected in the deciduous species due to the switch in respiratory C supply from recent photosynthates to storage carbon during winter, similar to observations during germination of *Phaseolus vulgaris* (Bathellier *et al.*, 2008). Consequently, the seasonal dynamics were expected to be damped in the evergreen species, which may profit from photosynthates formed during mild winter periods.

Materials and methods

Experimental Site

The study was conducted at the forest research site Kranzberger Forst (48°25’ 08’’ N, 11°39’ 41’ E, 485 m a.s.l., Freising, Germany). The mixed forest consisted of 60 - 70 year old individuals of the late-successional tree species European beech (*F. sylvatica*) and Norway spruce (*P. abies*), respectively. Beech trees were established in dense groups of 50 - 60 individuals (Pretzsch *et al.* 1998). The mean tree height was 28.8 ± 0.8 m in spruce and 25.9 ± 0.3 m in beech (Table 2.1). Stand density was 829 trees ha^{-1}, and basal area amounted to 46.4 m^2 ha^{-1} (Wipfler *et al.*, 2005). The long-term averages (1970 - 2000) of annual mean air temperature and precipitation were 7.8 ℃ and 786 mm, respectively (Matyssek *et al*., 2007). The dominant soil type was Luvisol with high nutrition and water supply. Slope inclination was orientated North, not exceeding 1.8°. Tree canopies were accessible by scaffolding between 17 to 25 m aboveground and by a canopy crane (Häberle *et al.*, 2004). The current study was conducted on seven adult individuals each of beech and spruce from May 2008 through June 2009.

Meteorological parameters

Air temperature (T_{Air}), solar radiation, air pressure, and absolute air humidity were measured above the canopy on top of the scaffolding. Precipitation was assessed at a forest climate station of the LWF (Bayerische Landesanstalt für Wald und Forstwirtschaft, Freising, Germany), approximately 1 km distant from the research site.

The mean annual T_{Air} during the experiment was 8.2 ± 0.4 ℃ (± SE, n = 365) and the sum of annual rainfall amounted to 724.4 mm (both calculated from 20 May 2008 through 20 May 2009, see also Fig. 2.1). Compared to long-term observations (1970 – 2000) T_{Air} was higher by 0.4 ℃ and precipitation lower by 62 mm. Winter snow cover during the study period lasted from December 2008 until mid March 2009.

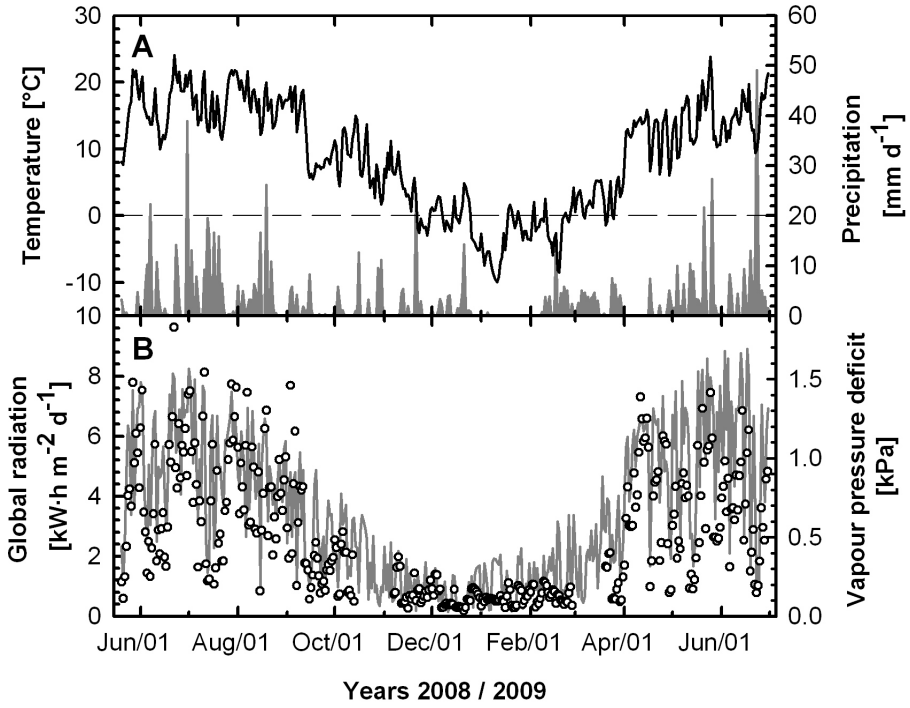


Fig. 2.1: Weather conditions throughout the experiment. **A**: Mean daily temperature (black line) and sum of precipitation (gray area), **B**: Sum of global radiation per day (gray line) and mean daily vapor pressure deficit (open circles). ·

CO_2 efflux rate

The CO_2 efflux rate of both upper and lower trunk and of one coarse root was determined on each study tree. In addition, CO_2 efflux was measured on one non-leafy branch per beech tree (n = 5). Plexiglass® (Röhm GmbH, Darmstadt, Germany) chambers were permanently installed at each

position (Table 2.1), resulting in a total of 47 chambers (beech *n* = 26, spruce *n* = 21) (for details see Grams *et al*., 2011). Trunk chambers were laterally attached, enclosing a trunk surface area of 203 to 534 cm^2. Roots and branches were completely enclosed by chambers. Each chamber was equipped with a NTC temperature sensor (negative temperature coefficient sensor, Pt 100, SEMI 833 ET, Hygrotec® Messtechnik GmbH, Titisee, Germany) installed 5 mm into the bark to measure cambial temperature (T_{Bark} in ℃). All chambers were covered with aluminized polyester foil.

Table 2.1: Tree size, height of CO_2 efflux chambers, diameter of corresponding tree component and sap wood depth behind chambers (means ± SE).

Species	Position within tree	Height above ground (m)	Diameter (cm)	Sapwood depth (cm)	Sapwood volume (cm^3)
Beech	Tree size	25.9 ± 0.3	-/-	-/-	-/-
	Branch	22.6 ± 1.0	1.7 ± 0.1	0.8 ± 0.0	35.9 ± 7.9
	Upper trunk	12.6 ± 2.4	17.9 ± 3.9	6.6 ± 0.9	1063.6 ± 249.7
	DBH	1.3 ± 0.0	24.6 ± 3.5	8.5 ± 0.7	-/-
	Lower trunk	0.7 ± 0.1	25.5 ± 3.5	8.8 ± 0.9	2935.6 ± 433.1
	Root	-0.08 ± 0.02	1.5 ± 0.1	0.7 ± 0.0	18.1 ± 2.7
Spruce	Tree size	28.8 ± 0.8	-/-	-/-	-/-
	Upper trunk	6.3 ± 0.1	27.5 ± 2.0	6.1 ± 0.7	1539.9 ± 319.8
	DBH	1.3 ± 0.0	33.7 ± 2.4	7.7 ± 0.5	-/-
	Lower trunk	0.7 ± 0.1	34.9 ± 2.7	7.9 ± 0.9	2839.6 ± 113.1
	Root	-0.05 ± 0.01	1.6 ± 0.2	0.8 ± 0.1	27.9 ± 10.8

DBH = diameter breast height. Sample size *n* = 7 except Beech branches (*n* = 5)

CO_2 efflux rates were assessed by a computer-controlled flow-through gas-exchange system (Fig. 2.2a). A stable reference gas (406.6 ± 0.9 µmol CO_2 mol^{-1}; $\delta^{13}C$ = - 3.06 ± 0.02 ‰, mean ± SE, *n* = 325) was continuously pumped via PVC tubes through the chambers at a rate of approx. 0.5 l min^{-1}. Outgoing chamber air was analyzed by an IRGA (Infra-red gas analyzer, Binos 4b, Emerson Process Management, Weißling, Germany). Two parallel systems allowed for the simultaneous measurements of a maximum of 28 chambers, automatically interchanging between sampling chambers every 8 min. Measurements on beech and spruce were interchanged approximately every two weeks.

Trunk efflux rates were expressed per unit volume (m^{-3}) of living tissue (sapwood + bark, Maunoury *et al.*, 2007; Saveyn *et al.*, 2008). Sapwood depth was assessed at the end of the experiment on wood cores taken next to the chambers. Branch and coarse root efflux rates were related to the total enclosed organ volume (Desrochers *et al.*, 2002; Marsden *et al.*, 2008).

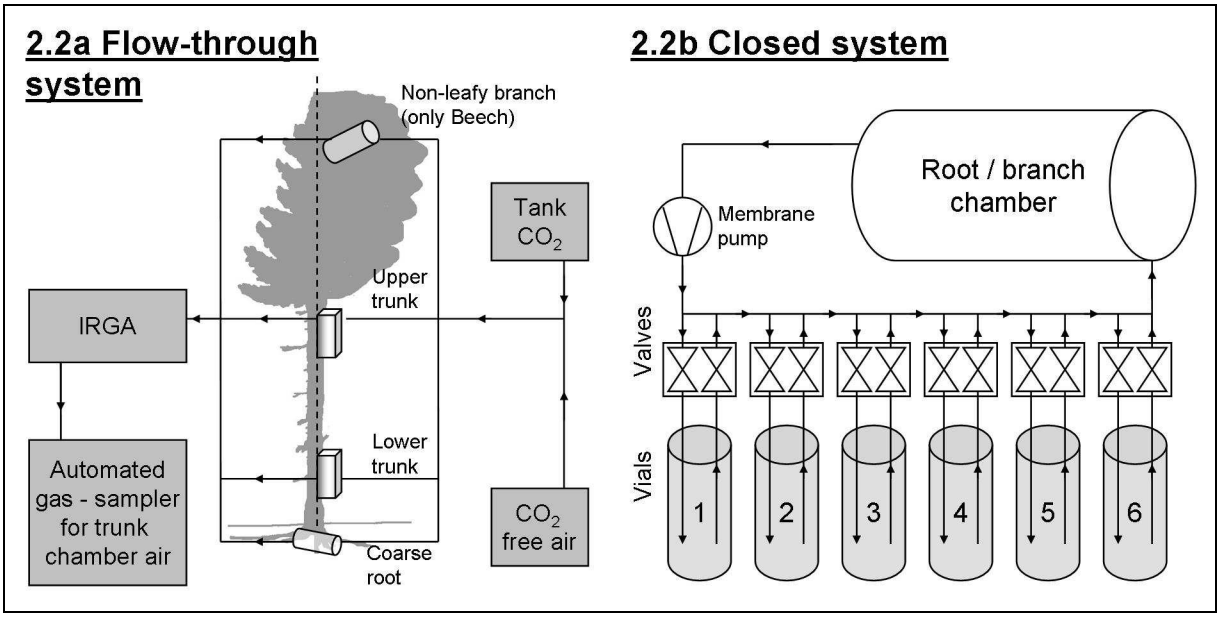


Fig. 2.2: Experimental setups for the assessment of CO_2 efflux rate and the $\delta^{13}C$ of CO_2 efflux ($\delta^{13}C_E$). **a**: Flow-through system for assessment of CO_2 efflux rates of all studied tree components (branch, upper trunk, lower trunk and coarse root) and sampling of trunk chamber air for determination of $\delta^{13}C_E$. **b**: Closed system for assessment of $\delta^{13}C_E$ of non-leafy branches and coarse roots.

Growth and maintenance respiration

Maintenance respiration (R_M) was assessed using the mature-tissue method (Amthor, 1989; Ceschia *et al.*, 2002; Wieser *et al.*, 2009). Respiration rate (R) was assumed to equal CO_2 efflux and related to bark temperature according to Eqn. 2.1:

$$R = R_{15} * Q_{10}^{\frac{T_{Bark} - 10}{15}} \quad (\mu mol * m^{-3} * s^{-1}), \qquad \text{Eqn. 2.1}$$

whereby R_{15} represents the efflux rate at 15 °C and Q_{10} the temperature sensitivity of the CO_2 efflux. It was assumed that R equals R_M during periods without growth (Amthor, 1984) and accordingly, we determined R_{15} and Q_{10} during winter (November through February) individually for each chamber and week. Individual Q_{10} and R_{15} were used to calculate R_M for the whole year on the basis of T_{Bark}. We are aware that CO_2 efflux may not necessarily represent the actual respiration due to possible contributions of CO_2 transported within the xylem sap (Teskey *et al.*, 2008). However, regarding potential CO_2 contamination of the respiration rate by the sap flow, the coefficient of determination between CO_2 efflux and sap flow were low (general linear model: $R^2_{beech} = 0.18$, $R^2_{Spruce} = 0.07$, both p

≤ 0.001, n > 4900, each, see also discussion). The calculated difference between R_M and the actual CO_2 efflux rate was used to identify periods of active growth respiration (R_G).

CO_2 sampling for stable carbon isotope analysis

The stable C isotope composition ($\delta^{13}C$) of the CO_2 efflux was measured once a month in beech and spruce throughout the whole experiment (i.e. 13 months). Beech trees were monitored from May 2008 through May 2009 and spruce from June 2008 through June 2009. All chambers were leak-tested one week before each sampling by applying a slight over-pressure (ca. 2000 Pa) while tolerating pressure drops of < 100 Pa min^{-1}. In the case of trunk chambers, the stable carbon isotope ratio of the CO_2 efflux was calculated using a two-end-member mixing model (see Dawson *et al.*, 2002). To this end, both the $\delta^{13}C$ and the $[CO_2]$ of chamber and reference air were sampled ($\delta^{13}C_{sample}$, $[CO_2]_{sample}$, $\delta^{13}C_{reference}$ and $[CO_2]_{reference}$, respectively). The $\delta^{13}C$ of CO_2 efflux ($\delta^{13}C_E$) was then calculated as:

$$\delta^{13}C_E = \frac{\left([CO_2]_{sample} * \delta^{13}C_{sample}\right) - \left([CO_2]_{reference} * \delta^{13}C_{reference}\right)}{[CO_2]_{sample} - [CO_2]_{reference}} \quad (‰), \qquad \text{Eqn. 2.2}$$

An automated gas sampler (Gilson 221 XL, Gilson Inc. Middleton, USA) sampled both chamber and reference air into 12 ml glass vials (Exetainer, Labco Limited, High Wycombe, UK) after the IRGA of the flow-through gas-exchange system (see Fig. 2.2a). Samples were then analysed for $\delta^{13}C$ by IRMS (isotope-ratio mass spectrometry, GVI-Isoprime, Elementar, Hanau, Germany). Previous testing at individual points of the system revealed that the whole flow-through gas exchange system (including the IRGA) had no contaminating effect on $\delta^{13}C_{Reference}$ (see Grams *et al*., 2011). Each trunk chamber was sampled every 3 h.

The amount of CO_2 released from coarse roots and non-leafy branches was too small to be analyzed by the flow-through systems. Instead $\delta^{13}C_E$ was assessed by means of a custom-built closed-respiration system that included a total of six sampling vials (Fig 2.2b, cf. Prater *et al*. 2006). For sampling, the system was operated in a closed mode for about one hour, and CO_2 release from the coarse root or branch resulted in an increase of $[CO_2]$. About every 10 minutes one sampling vial was isolated by simultaneously closing valves directly in front of and behind the vial. To obtain reliable estimates of $\delta^{13}C_E$, we adjusted the sampling interval to attain a $[CO_2]$ range of ≥ 100 µmol mol^{-1}

between the first and last sample. The $\delta^{13}C$ of CO_2 efflux was calculated from the six gas samples taken at each measurement according to the "Keeling Plot Approach" (Keeling, 1958, Zobitz *et al.*, 2008). Keeling plots with coefficients of determination (R^2) < 0.97 were rejected. Root and branch $\delta^{13}C_E$ were sampled twice a day during early morning and onset of night.

Sap flow measurements

Sap flow was assessed for each tree at a height of 1.3 m. First, sap flow density was assessed using two Granier-type sensors per tree (Granier, 1985) installed into trunks at 2 cm depth. Decline functions of sap flow density towards central sapwood parts (> 2 cm) were available for the study site (U. Metzger, Ecophysiology of Plants, Technische Universität München, pers. com.) and used to scale measured sap flow density to whole-tree sap flow. Sapwood depth was assessed on wood cores next to sensors.

Leaf net CO_2 exchange

The net rate of leaf CO_2 exchange (J_{CO2} in mmol CO_2 m^{-2} leaf area d^{-1}) was measured on one sun branch per species with two permanently installed gas exchange chambers (Götz, 1996). The CO_2 concentration at the chamber inlet and outlet was assessed each by an IRGA (BINOS 4b.2, Emerson Process Management, Weißling, Germany). Net CO_2 exchange rates were related to the projected leaf area and calculated according to von Caemmerer and Farquhar (1981).

Statistical analysis

All data were analyzed using SPSS 16.0 (SPSS GmbH Software, Munich, Germany). Paired and independent Student's t-Tests were used to identify significant differences in mean $\delta^{13}C_E$ between two consecutive measurements at the same position (branch, upper and lower trunk, coarse root), or between species / sampling positions during leaf fall and leaf flush. For longer time periods, i.e. the main vegetative period and the winter dormancy, this was analyzed by repeated measures analysis of variance. General linear models (GLM) were used to explain the amount of variance for the whole seasonal patterns in $\delta^{13}C_E$. Covariates used were T_{Bark}, sap flow and CO_2 efflux rate (the latter only in the case of lower trunk $\delta^{13}C_E$). The day of the year (*DOY*) and the tree individual (*TI*) were treated as constant factors.

Results

CO_2 efflux, sap flow and net photosynthetic rate

In general, CO_2 efflux of all tree components, sap flow and J_{CO2}, followed the annual course of T_{Air} (Fig 2.1, Fig 2.3). In beech, branch CO_2 efflux peaked at the end of May, followed consecutively by upper trunks (mid-July), lower trunks and coarse roots (both end of July). Similarly, the CO_2 efflux in spruce displayed maxima at upper and lower trunks during mid-June followed by roots at the end of July. CO_2 efflux rates of both species were similar, except for lower trunks during summer and for coarse roots in early spring. In both cases, spruce CO_2 efflux was nearly twice as high as in beech. R_M followed a similar annual course as the CO_2 efflux. Growth respiration (R_G = differences between the CO_2 efflux rate and R_M) was observed from mid-May through mid-October, indicating active growth processes in the tree components during this time (Fig. 2.3).

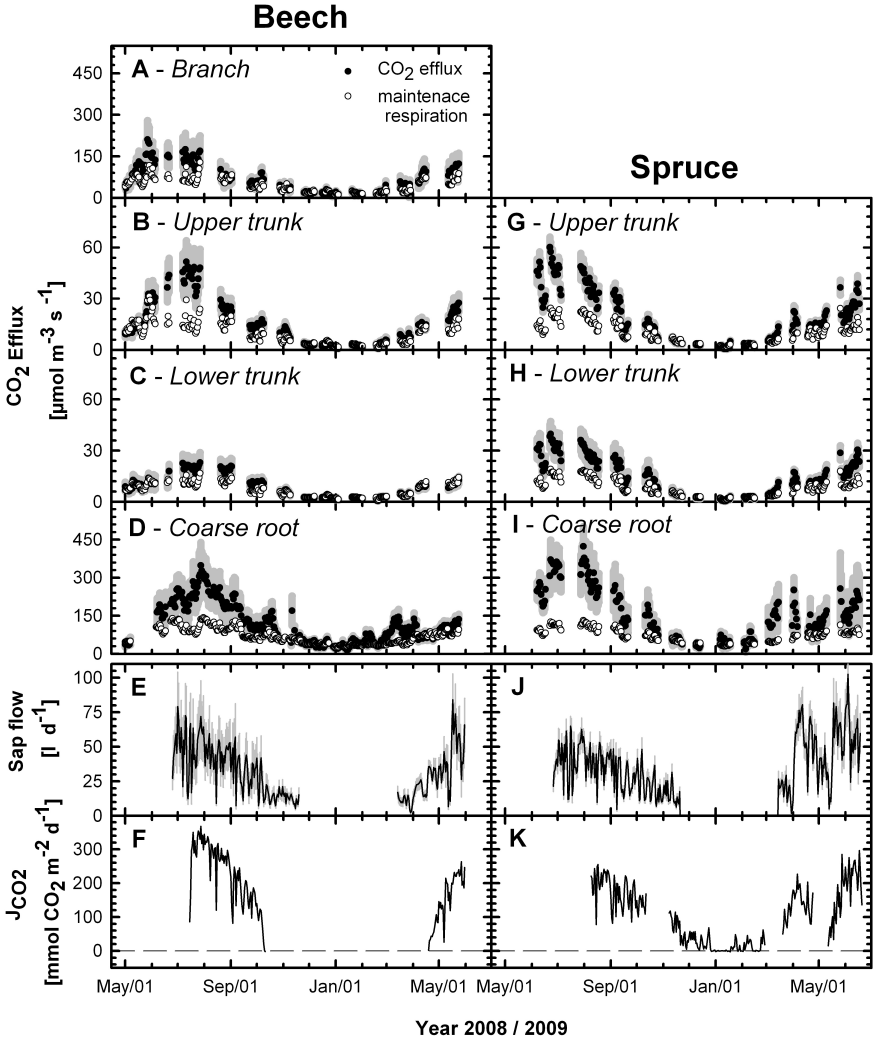


Fig. 2.3: Daily means of CO_2 efflux (closed circles) at different sample positions, sap flow and leaf net CO_2 exchange rate for beech (**A - F**) and spruce (**G – K**) throughout the experiment. Open circles give estimates of maintenance respiration. Gray areas give standard errors (SE). **A**: branch, **B + G**: upper trunk, **C + H**: lower trunk, **D + I**: coarse root. **E + J**: Daily sap flow. **F + K**: Rate of leaf net CO_2 exchange (J_{CO2}).

Both species had comparable rates of tree sap flow. Only during April and May 2009, spruce sap flow rate was higher compared to beech. During summer, J_{CO2} in spruce was lower than in beech. However, during winter, significant periods of leaf net CO_2 uptake were observed in spruce.

Intra- and interspecific differences in $\delta^{13}C_E$

No significant within-species differences were found between $\delta^{13}C_E$ of non-leafy branches and upper trunks or between upper and lower trunks (Fig. 2.4, Table 2.2a). During leaf flush and leaf fall, the $\delta^{13}C_E$ of beech coarse roots was enriched in ^{13}C compared to lower trunks. During the main vegetative period, spruce coarse roots were significantly enriched in ^{13}C compared to lower trunks (Tab 2.a). The opposite was the case during winter dormancy (for more details see Fig. 2.6).

The two species displayed significant differences in $\delta^{13}C_E$ of the same tree components (Fig. 2.5, Table 2.2b). The $\delta^{13}C_E$ of spruce trunks was enriched in ^{13}C compared to beech, except for the upper trunk position during winter. The same trend was observed in coarse roots.

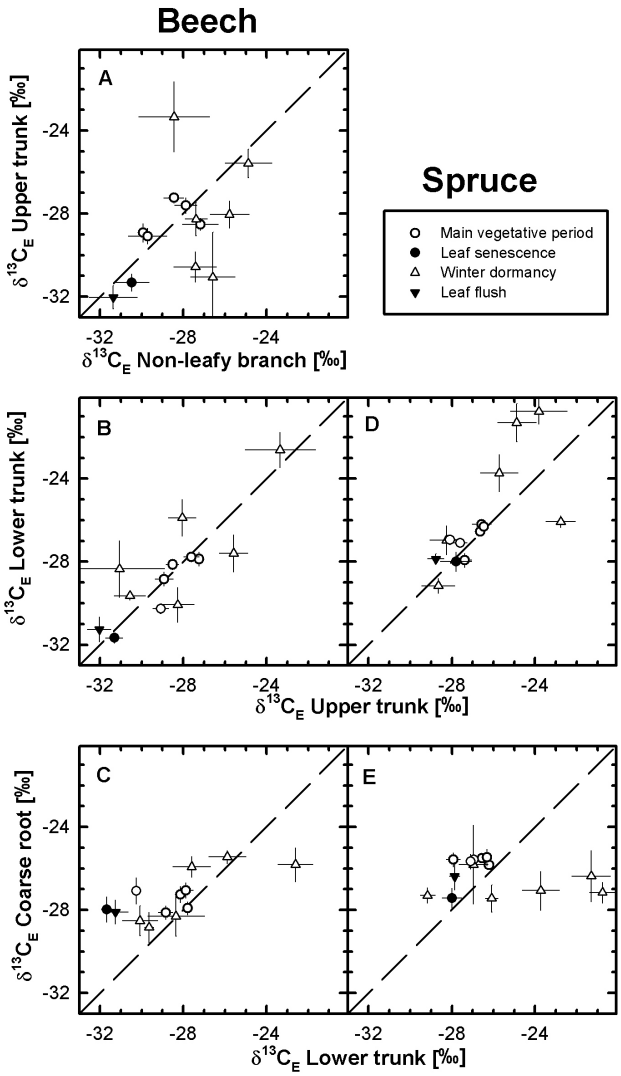


Fig. 2.4: Intraspecific comparison in $\delta^{13}C$ of CO_2 efflux ($\delta^{13}C_E$) between sample positions for beech and spruce at different phenological stages. Dashed lines indicate the one-to-one relationship. Sample size *n* per data point = 10 – 14.

Table 2.2: Differences in $\delta^{13}C$ of CO_2 efflux between two sample positions ($\delta^{13}C_B$ = branch, $\delta^{13}C_{UT}$ = upper trunk, $\delta^{13}C_{LT}$ = lower trunk and $\delta^{13}C_R$ = coarse root) within the same species (**A**) and between species (**B**).

A Difference in $\delta^{13}C$ within one species

Species	Period	$\delta^{13}C_B - \delta^{13}C_{UT}$	$\delta^{13}C_{UT} - \delta^{13}C_{LT}$	$\delta^{13}C_{LT} - \delta^{13}C_R$
Beech	I Leaf flush[1]	0.7 ± 1.2	-0.8 ± 0.8	-3.2 ± 0.8***
	II Main vegetative period[3]	-0.4 ± 0.3	0.1 ± 0.3	-0.6 ± 0.2
	III Leaf senescence (beech)[1]	0.8 ± 0.8	0.4 ± 0.5	-3.7 ± 0.7***
	IV Winter dormancy[3]	0.7 ± 0.7	-0.3 ± 0.7	-0.1 ± 0.6
Spruce	I Leaf flush[1]	-/-	-0.9 ± 0.5	-1.5 ± 0.7
	II Main vegetative period[3]	-/-	-0.0 ± 0.2	-1.1 ± 0.2**
	III Leaf senescence (beech)[1]	-/-	0.2 ± 0.8	-0.6 ± 0.7
	IV Winter dormancy[3]	-/-	-0.5 ± 0.7	2.4 ± 0.6*

B Difference in $\delta^{13}C$ between spruce and beech $\delta^{13}C_{spruce} - \delta^{13}C_{beech}$

	Period	$\delta^{13}C_{UT}$	$\delta^{13}C_{LT}$	$\delta^{13}C_R$
	I Leaf flush[2]	3.3 ± 0.8**	3.4 ± 0.9***	1.8 ± 0.9
	II Main vegetative period[3]	1.3 ± 0.3***	1.6 ± 0.2***	1.8 ± 0.6***
	III Leaf senescence (beech)[2]	3.5 ± 0.6***	3.7 ± 0.5**	2.4 ± 1.0*
	IV Winter dormancy[3]	2.4 ± 0.9	2.5 ± 0.9**	0.4 ± 0.3

Significant differences were calculated by (1) paired Student's t-Test, (2) independent Student's t-Test and (3) repeated measures analysis of variance for the whole phenological period. Data are shown as mean differences ± SE. Significance levels of $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$ are represented by *, ** and ***. Sample size *n* for period (I) and (III) = 10 - 14, for period (II) = 40 – 56 and for period (IV) = 60 - 84.

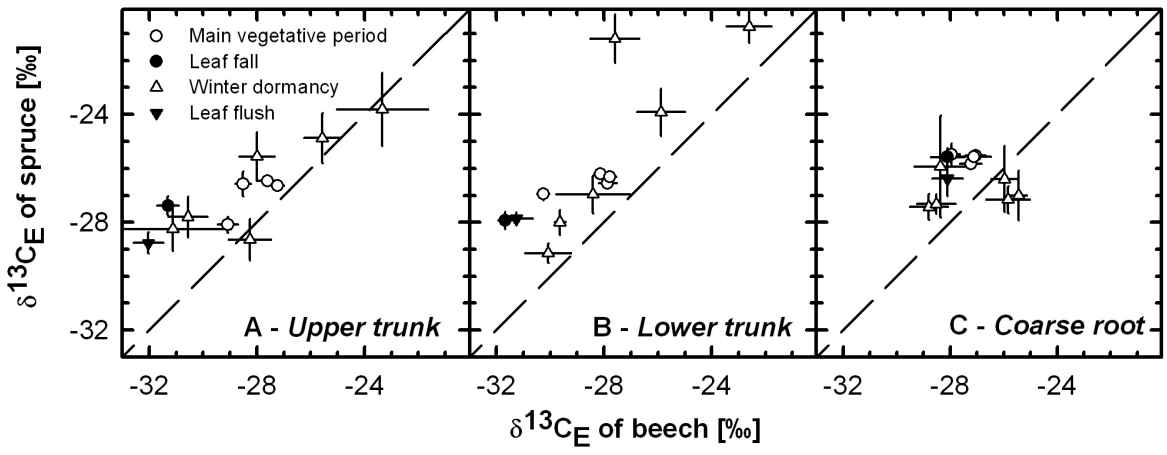


Fig. 2.5: Interspecific comparison in $\delta^{13}C$ of CO_2 efflux ($\delta^{13}C_E$) of beech (x-axis) and spruce (y-axis) per month. **A** = upper trunk, **B** = lower trunk, **C** = coarse root. Dashed lines indicate the one-to-one relationship. Sample size *n* per data point = 14.

Seasonal dynamics in $\delta^{13}C_E$

In both species, distinct seasonal changes in $\delta^{13}C_E$ were observed (Fig. 2.6). Changes in $\delta^{13}C_E$ of non-leafy branches and trunks were most pronounced outside of the main growing season, whereas $\delta^{13}C_E$ of coarse roots was generally less dynamic (Fig. 2.6).

Upon completion of leaf flush in April (period I), daily means of $\delta^{13}C_E$ for beech branches, upper trunks and lower trunks were rather stable throughout the vegetative period (II) (-28.2 ± 0.2 ‰, -28.1 ± 0.4 ‰ and -28.4 ± 0.2 ‰, respectively, see Table 2.3), but they significantly declined during leaf senescence (period III, -30.5 ± 0.8 ‰, -31.1 ± 0.4 ‰ and -31.7 ± 0.3 ‰, respectively). From this minimum onwards, a steady increase occurred throughout the winter dormancy (period IV, by 5.6 ‰, 8.0 ‰ and 9.1 ‰, respectively). Except for branches, this increase was significant over the whole period (see repeated measures analysis of variance in Table 2.3) and was followed by a distinct decline at the beginning of leaf flush (period I, -6.5 ‰, -9.0 ‰ and -8.7 ‰, respectively). Values observed during leaf flush were similar to those during leaf fall of the previous year. The observed decline in spring occurred first in branches (March 2009). In contrast to above-ground organs, coarse root $\delta^{13}C_E$ of beech was less dynamic but nevertheless significantly increased during winter dormancy (period IV, from -28.3 ± 1.0 ‰ to -25.4 ± 0.3 ‰, Fig. 2.6, Table 2.3).

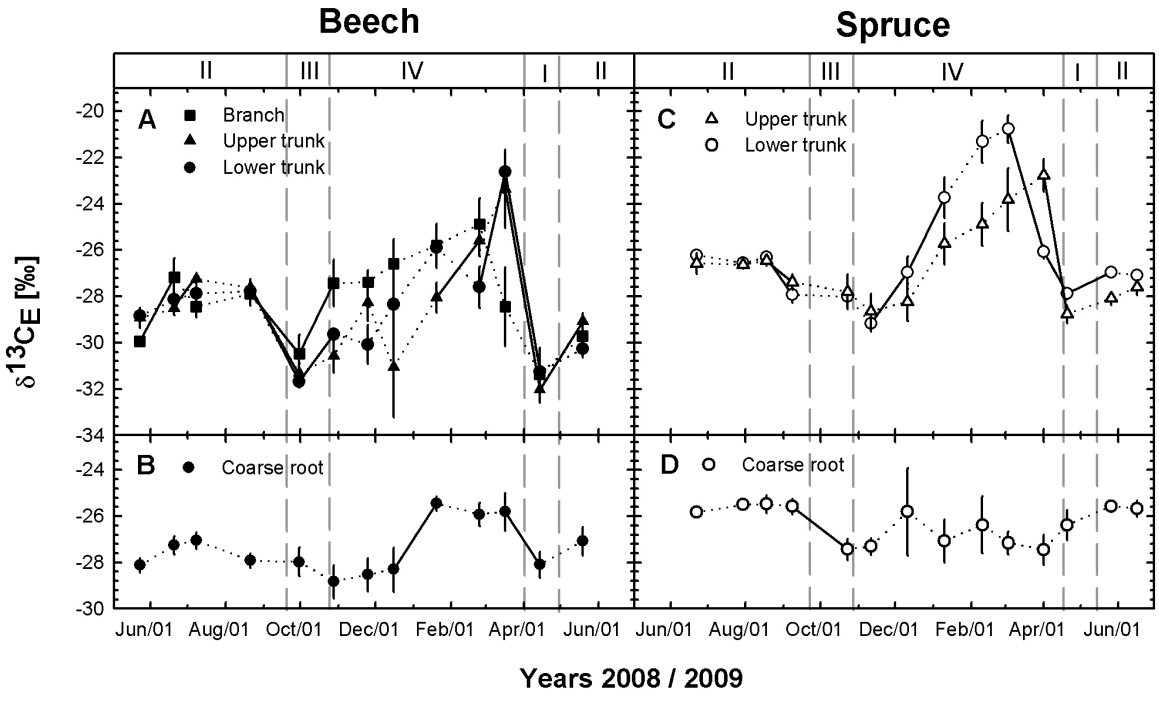


Fig. 2.6 Annual changes in $\delta^{13}C$ of CO_2 efflux ($\delta^{13}C_E$) of branches, trunks and coarse roots of beech (closed symbols, **A + B**) and spruce (open symbols, **C + D**). Data give daily means ± SE. Sample size for each data point n = 10, 56 and 12 for branches, trunks and coarse roots, respectively. **A** and **C** give $\delta^{13}C_E$ of above-ground organs. **B** and **D** give $\delta^{13}C_E$ of coarse roots (circle). Significant differences between two consecutive data points are marked by continuous lines (with at least $*p \leq 0.05$). Vertical lines separate phenological stages: I – leaf flush, II – main vegetative period, III – leaf senescence of beech, IV – winter dormancy.

In spruce, the decline in $\delta^{13}C_E$ of above-ground organs during leaf fall was smaller than in beech (period III). The $\delta^{13}C_E$ of spruce lower trunks increased stronger during winter dormancy (period IV) than in upper trunks (8.4 ‰ and 5.9 ‰, respectively).

Table 2.3: Influence of the time point of measurement on the $\delta^{13}C$ of branch, trunk and coarse root CO_2 efflux of beech and spruce trees, detected with repeated measures analysis of variance.

Species	Sample position	Main vegetative period (II)	Winter dormancy (IV)
Beech	Branch	$p = 0.057$	$p = 0.619$
	Upper trunk	$p = 0.112$	**$p = 0.046$**
	Lower trunk	**$p = 0.018$**	**$p = 0.002$**
	Coarse root	$p = 0.111$	**$p = 0.018$**
Spruce	Upper trunk	$p = 0.381$	**$p = 0.024$**
	Lower trunk	**$p \leq 0.001$**	**$p \leq 0.001$**
	Coarse root	$p = 0.814$	$p = 0.815$

Significance levels are shown as p - values. Bold type mark all $p \leq 0.05$. Sample size n = 20 - 28 for period (II) and 30 - 42 for period (IV).

In both species, the variance in $\delta^{13}C_E$ of upper and lower trunks was found to be significantly related to the day of the year (*DOY*, strongest influence), the tree individual (*TI*), T_{Bark} and the CO_2 efflux rate (Table 2.4). Sap flow did not cause a significant effect on $\delta^{13}C_E$. Including all parameters within one model explained a high amount of variance (R^2 upper trunk: beech: 0.74, spruce: 0.70, R^2 lower trunk: beech: 0.83, spruce: 0.59, all with $p \leq 0.001$, $n > 350$). Again, *DOY* had the strongest influence.

Table 2.4: Influence of different parameters on annual variability in $\delta^{13}C$ of trunk CO_2 efflux, given by their coefficients of determinants.

Species	Position	*DOY*	*TI*	T_{Bark}	CO_2 efflux	Sap flow
Beech	Upper trunk	**0.61*** **	**0.10*** **	0.00	**0.02***	-
	Lower trunk	**0.66*** **	**0.07*** **	**0.02****	0.00	0.00
Spruce	Upper trunk	**0.53*** **	**0.04*** **	**0.04*** **	0.00	-
	Lower trunk	**0.59*** **	0.01	**0.12*** **	**0.04*** **	0.00

Parameters were tested individually by GLM. *DOY* = Day of the year, *TI* = tree individual, T_{Bark} = bark temperature. Bold type indicates a significant correlation. Significance levels of $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$ are represented by *, ** and ***. Sample size $n > 350$ per trunk position.

Discussion

The main objective of the present study was to identify seasonal dynamics in $\delta^{13}C_E$ for a deciduous (*F. sylvatica*) and an evergreen (*P. abies*) European tree species. Distinct changes in $\delta^{13}C_E$ were recorded between summer and winter periods. The most pronounced seasonal dynamics were found in deciduous Beech during phenological changes and winter dormancy.

Contribution of sap flow to $\delta^{13}C_E$

In addition to local tissue respiration, CO_2 transported with the xylem sap was recently recognized to contribute to CO_2 efflux (Hölttä & Kolari, 2009, Saveyn *et al.*, 2008, Teskey *et al.*, 2008). Plant-respired and soil CO_2 may enter the xylem stream and be transported up the tree (up to 60% and 10%, respectively, see Moore *et al.*, 2008; Teskey *et al.*, 2008; Aubrey & Teskey, 2009). In the present study, only a weak positive correlation between rate of sap flow and CO_2 efflux was observed in trunks (beech R^2: 0.18, spruce: R^2: 0.07 both $p \leq 0.001$). Moreover, $\delta^{13}C_E$ was not significantly correlated with sap flow (Table 2.3). Thus, we conclude that in our study the $\delta^{13}C_E$ in European beech and Norway spruce was not affected by CO_2 transported with the xylem sap, which is in accordance with recent findings (Grams *et al*. 2011; Ubierna *et al.*, 2009).

Seasonality in $\delta^{13}C$ of beech CO_2 efflux

After the completion of leaf flush (period I), $\delta^{13}C_E$ of above-ground organs was rather stable during times of growth respiration (main vegetative period (II): -28.3 ± 0.2 ‰) and significantly declined during leaf senescence (period III: -31.2 ± 0.4 ‰). During period (II), large fractions of recent photosynthates are used for wood formation, i.e. lignin and cellulose synthesis, whereas in September, photosynthates are assumed to be mainly used for the build-up of reserves, such as starch (Barbaroux & Breda, 2002, Helle & Schleser, 2004, Vaganov *et al.*, 2009). Starch is enriched in ^{13}C compared to phloem sugars (up to 4 ‰, see Bowling *et al*., 2008) due to fractionation processes during its synthesis (Gessler *et al*., 2008; Tcherkez *et al.*, 2004). Consequently, during starch accumulation, the remaining respiratory substrate and the respired CO_2 are assumed to be relatively ^{13}C depleted (Maunoury *et al*. 2007). The observed drop at the end of the growing season from about -28.3 to -31.2 ‰ fits to this consideration and could be interpreted as a switch from woody tissue formation to the accumulation of storage C.

During winter dormancy (period IV), we observed a steady increase in $\delta^{13}C_E$ of branches and trunks. This may partly be attributed to a switch of respiratory C supply from recent photosynthates to storage C, i.e. from sugars to more ^{13}C enriched starch (Damesin & Lelarge, 2003, Maunoury *et al.*, 2007). Because starch is typically 1 - 2 ‰ enriched in ^{13}C compared to sugars (Bowling *et al*., 2008), this switch alone is unlikely to account for the observed increase in $\delta^{13}C_E$ of 5.6 - 9.1 ‰. We note that starch may become continuously ^{13}C enriched during winter, but we are not aware of a corresponding biochemical mechanism for this. On the other hand, the TCA cycle is often assumed to be sensitive to low temperatures, in particular in comparison to PDH activity (Atkin *et al.*, 2000, Kodama *et al.* 2008). Thus, prevailing PDH activity during winter may release ^{13}C enriched CO_2 (Maunoury *et al.*, 2007, Priault *et al.*, 2009, Tcherkez *et al.*, 2003). However, $\delta^{13}C_E$ was not correlated to T_{Bark} during winter dormancy (data not shown) and reached its highest levels in March when temperatures were already rising. Consequently, the slow but constant increase of $\delta^{13}C_E$ is unlikely caused only by the temperature dependent metabolic branching between TCA and PDH activity alone. Recently, high phospho*enol*pyruvate carboxylase (PEPc) activity has been demonstrated in trunks (Berveiller & Damesin, 2008). In this reaction, PEPc re-fixes respired CO_2 to supply the TCA cycle with ^{13}C enriched malate (Berveiller *et al*., 2007b), favoring $^{13}CO_2$ by 5.7‰ (Raven & Farquhar, 1990). While TCA cycle activity is low, PEPc derived malate may not be metabolized completely and may accumulate in trunks (Gessler *et al*., 2009b). This may gradually enrich the respiratory substrate pool in ^{13}C as this malate may be metabolized in the TCA cycle. To address this hypothesis, future research should focus on the simultaneous measurements of $\delta^{13}C_E$ and respiratory substrate pools in trunks during winter. During leaf flush (period I) we observed a rapid decrease in $\delta^{13}C_E$ to a minimum of about -31.6 ‰, similar to levels during leaf senescence (period III). This suggests that the pool of malate was already completely metabolized at rising temperatures (Barbour *et al*., 2007). Additionally, soluble C reserves (i.e. sugars and transitory starch) may be mobilized and allocated from lower tree parts to branches (Lacointe *et al*., 2004), while in the trunk, ^{13}C depleted lipids may serve as respiratory substrates (Tcherkez *et al.*, 2003).

Conversely, coarse root $\delta^{13}C_E$ of beech was relatively stable throughout the year and increased during winter dormancy only by about 2.8‰. Our data from adult forest trees are consistent with results from Bathellier *et al*. (2009) who observed $\delta^{13}C_E$ of root respiration in *Phaseolus vulgaris* to be almost constant (about -27.5 ‰), even under C starvation (i.e. 4 days of continuous darkness). According to model calculations, this was due to a high contribution of the pentose phosphate pathway

(PPP) to root $\delta^{13}C_E$ (up to 25%, Bathellier *et al.*, 2009, Dieuaide-Noubhani *et al.*, 1995). Like the TCA cycle, PPP produces ^{13}C depleted CO_2. Under starvation, the relative contribution of the TCA cycle changes, accompanied by the use of different respiratory substrate, i.e. lipids / proteins. However, while this may affect the $\delta^{13}C_E$ of leaf respired CO_2 (Tcherkez *et al.*, 2003), respective changes in roots are damped by PPP derived CO_2 (Bathellier *et al.*, 2009). As opposed to other findings (Klumpp *et al.*, 2005, Schnyder & Lattanzi, 2005) root CO_2 efflux was not depleted in ^{13}C compared to trunks and branches (Table 2a). However in the present study, root $\delta^{13}C_E$ originates from coarse roots (diameter of about 1.5 cm) and does not reflect respiration of fine roots or whole root systems as it is the case in the above mentioned studies.

Differences between spruce and beech

Compared with beech, the $\delta^{13}C_E$ of spruce was consistently enriched in ^{13}C. While we are not aware of studies comparing $\delta^{13}C_E$ from a conifer with that of a deciduous tree species, Garten & Taylor (1992) observed a similar offset in foliar $\delta^{13}C$. Likewise, soil CO_2 efflux near conifers was reported to be enriched in ^{13}C compared with soils under broad-leafed trees (Andersen *et al.*, 2010, Steinmann *et al.*, 2004). This difference most likely results from a lower ci/ca ratio in conifer needles compared to leaves of deciduous species, resulting in a lower photosynthetic discrimination against $\delta^{13}C$ (Farquhar *et al*., 1989).

Compared with beech, the drop of $\delta^{13}C_E$ in spruce during fall (period III) was less pronounced and may relate to less starch synthesis in spruce compared to beech. The increase of $\delta^{13}C_E$ in the upper trunk during winter (period IV) was less pronounced than in the lower part of the trunk. We hypothesize that upper trunk respiration was partially fed by winter photosynthates, as spruce needles displayed prolonged periods with positive net photosynthesis during winter (Fig. 2). Likewise in beech, ^{13}C enrichment of the CO_2 efflux in branches was smaller than in trunks (i.e. about 5.9 versus 8.0 – 9.1 ‰). In beech branches, autotrophic C supply to respiration may result from corticular photosynthesis, producing photosynthates during mild winter days (Berveiller *et al.*, 2007a, Pfanz *et al.*, 2002). These photosynthates are mainly build by carboxylation of respired, ^{13}C depleted CO_2 (Pfanz *et al.*, 2002). Since all chambers were darkened with silver foil, branch $\delta^{13}C_E$ was not biased by corticular photosynthesis in chambers directly, but photosynthetic refixation could have occurred in the surrounding tissue. These ^{13}C depleted photosynthates could have been transported via the phloem along branches and respired into the chambers. The early decrease of branch $\delta^{13}C_E$ (compared to

trunks) at the end of the winter dormancy under rising temperatures support the idea that corticular photosynthesis contributes to respiratory C pools in beech branches.

In conclusion, strong and similar seasonal dynamics in $\delta^{13}C_E$ of above-ground plant components were observed in beech and spruce. The most pronounced changes in trunks were observed when growth respiration ceased and $\delta^{13}C_E$ was dominated by maintenance respiration (phase III, IV and I, Fig. 5). The observed seasonal patterns indicated a switch from woody tissue formation to starch accumulation at the end of trunk diameter growth. The large increase in $\delta^{13}C_E$ of 5.6 – 9.1 ‰ during winter dormancy could not completely be explained by remobilization of starch or by temperature sensitivity of the TCA cycle. We hypothesized an accumulation of ^{13}C enriched malate by PEPc, which needs to be tested in future research. Although seasonal dynamics were similar in both species, seasonal changes in $\delta^{13}C_E$ of beech were more pronounced, most likely due to a more abrupt switch in respiratory C supply from recent photosynthates to storage C in the deciduous species. Additional C supply by winter photosynthesis may explain the damped seasonal dynamics in $\delta^{13}C_E$ of non-leafy branches in beech and upper trunks in spruce. Overall, we conclude that the highest seasonal variation of $\delta^{13}C_E$ occurs in trunks that have no access to additional C from winter or corticular photosynthesis.

3. Seasonal pattern of carbon allocation to respiratory pools in 60-year-old deciduous (*Fagus sylvatica*) and evergreen (*Picea abies*) trees assessed via whole-tree stable carbon isotope labeling[2]

Summary

- CO_2 efflux of adult trees is supplied by both recent photosynthates and carbon (C) stores. The extent to which these C pools contribute to growth and maintenance respiration (R_G and R_M, respectively) remains obscure.
- Recent photosynthates of adult beech and spruce trees were labeled by exposing whole canopies to ^{13}C depleted CO_2. Label was applied three times during the year (spring, early and late summer) and changes in $\delta^{13}C$ of trunk and coarse root CO_2 efflux were quantified.
- Seasonal patterns in C translocation rate and fractional contribution of label to CO_2 efflux ($F_{Label\text{-}Max}$) were found. Phloem transport was fastest in early summer. In beech, $F_{Label\text{-}Max}$ peaked during early summer in coarse roots (0.4 ± 0.2) and during late summer in trunks (0.6 ± 0.3). No seasonal dynamics in $F_{Label\text{-}Max}$ were found in spruce.
- During spring, both R_G and R_M of beech were largely supplied by C stores. In early summer, R_G derived mainly from recent and R_M from stored C, whereas later in the season, both R_G and R_M were predominantly supplied by recent photosynthates. In spruce, R_G and R_M were constantly supplied by a mixture of stored C (ca. 75%) and recent photosynthates (ca. 25%).

[2] Submitted for publication as Kuptz D, Fleischmann F, Matyssek R, Grams TEE (2010) to *New Phytologist*

Introduction

Terrestrial carbon (C) fluxes are dominated by two major processes: Autotrophic C uptake by primary producers and ecosystem respiration (Gifford, 2003). In Europe, forests are the most abundant terrestrial ecosystems (44% of total land mass, FAO, 2006), fixing up to 3.25 Pg C $year^{-1}$ (gross primary productivity, Schultze *et al.*, 2010). Large amounts of the assimilated C are released back to the atmosphere by ecosystem respiration (Valentini *et al.*, 2000; Knohl *et al.*, 2003; Luyssaert *et al.*, 2010), divided into autotrophic plant respiration (on average 53% of GPP) and heterotrophic soil respiration (on average 33% of GPP, Schultze *et al.*, 2010).

Autotrophic respiration is usually measured as the CO_2 efflux from plants to the atmosphere and may be partitioned into growth respiration (R_G, related to processes such as diameter growth or cell wall thickening) and maintenance respiration (R_M, related to processes such as protein and lipid turnover or maintenance of the cellular structure, Amthor, 2000). For adult trees, large seasonal variations in the total CO_2 efflux and contribution by R_G and R_M were reported, with R_G mainly depending on growth activity and R_M on temperature (Stockfors & Linder, 1998; Ceschia *et al.*, 2002; Kuptz *et al.*, 2011b). CO_2 efflux may also be influenced by respiratory or soil CO_2 transported in the xylem (Teskey *et al.*, 2008). Up to date, large uncertainties remain on the origin of C in CO_2 efflux, in particular, on the age, number and magnitude of C sources (Trumbore, 2006; Carbone & Trumbore, 2007; Lehmeier *et al.*, 2008) and the extent to which they contribute to respiration (Lötscher *et al.*, 2004).

Throughout the growing season, recent photosynthates are often considered the most important C source for respiration (Scartazza *et al.*, 2004; Wertin & Teskey, 2008; Maier *et al.*, 2009). However, several recent reports on tree, herb and grassland respiration suggested that it's contributing C may be of variable age (Schnyder *et al.*, 2003; Nogues *et al.*, 2006). Photosynthates may supply respiration directly, but may also pass though storage pools with different half-lifes (Lehmeier *et al.*, 2008). Moreover, for sunflower (*Helianthus annuus*) and alfalfa (*Medicago sativa*), Lötscher *et al.* (2004) stated that R_G is supplied exclusively by recent photosynthates, whereas R_M is supplied by older C. In adult forest trees, previous studies observed fast intermixing of recent photosynthates with older substrate pools (Keel *et al.*, 2007; Kodama *et al.*, 2008). However, the extent to which these short- and long-term C pools contribute to R_G and R_M remains obscure.

Labeling of canopy air with stable C isotopes may unveil the fractional contribution of recent photosynthates to CO_2 efflux (Nogues *et al.*, 2006; Lehmeier *et al.*, 2008) and uncover the time lag

between photosynthetic C uptake and respiratory C loss (Högberg *et al.*, 2008; Plain *et al.*, 2009). Time lags between C uptake and respiratory loss may serve as an indicator of changes in source – sink relations of plant tissues and C transport rates should be high during times of active growth (Kozlowski, 1992; Wingate *et al.*, 2010). In the present study, we oriented our research at the findings by Lötscher *et al.* (2004), testing the following hypotheses on adult forest trees (I) that R_G of trunk and coarse roots is directly and exclusively supplied by recent photosynthates and (II) that R_M is feed by storage pool C. To this end, stable carbon isotope labeling of adult European beech (*Fagus sylvatica*, L.) and Norway spruce (*Picea abies*, Karst) was applied three times per year during times with contrasting contributions of R_G to CO_2 efflux.

Materials and Methods

Research site and meteorological data

The study was conducted at the forest research site Kranzberger Forst (48°25' 08'' N, 11°39' 41' E, 485 m a.s.l., Freising, Germany). Study species were European beech (*F. sylvatica*), forming dense groups of 50 – 60 individuals and Norway spruce (*P. abies*) growing in large parts of the area. Trees were 60 to 70 years old. For further details on the study site see Pretzsch *et al*. (1998). Long-term means (1970 – 2000) of annual air temperature and precipitation were 7.8℃ and 786mm, respectively (Matyssek *et al.*, 2007). Tree canopies were accessible by scaffolding (17 – 25 m above ground) and canopy crane.

Air temperature (T_{Air}), air pressure and absolute air humidity were continuously monitored at about 2 m above the canopy. Precipitation was assessed at a forest climate station of the LWF (Bayerische Landesanstalt für Wald und Forstwirtschaft, Freising, Germany), approximately 1 km distant from the research site.

Stable C isotope labeling

In 2008 and 2009, a series of six free-air $^{13}CO_2/^{12}CO_2$ labeling experiments were performed on adult individuals of beech and spruce. Four trees per species were labeled. Three unlabeled control trees of each species were sampled simultaneously. The stable C isotope composition of canopy air ($\delta^{13}C_{Air}$) surrounding each labeled tree was altered by the release of pure CO_2 ($\delta^{13}C_{Label}$ ca. -47‰) into the atmosphere, tolerating a [CO_2] increase of about 100 µmol/mol (see also Table 3.1). Labeling was

employed through the isoFACE approach, which allows for continuous, free-air $^{13}CO_2/^{12}CO_2$ labeling of adult tree canopies (Grams *et al.*, 2011). Briefly, tree canopies were equipped with micro-perforated PVC tubes for homogeneous CO_2 release. For beech trees, tubes were suspended from the stationary scaffolding at the research site. In the case of spruce, tubes were suspended from low-weight custom-build carrier structure attached to each labeled tree (see Fig. 3.1).

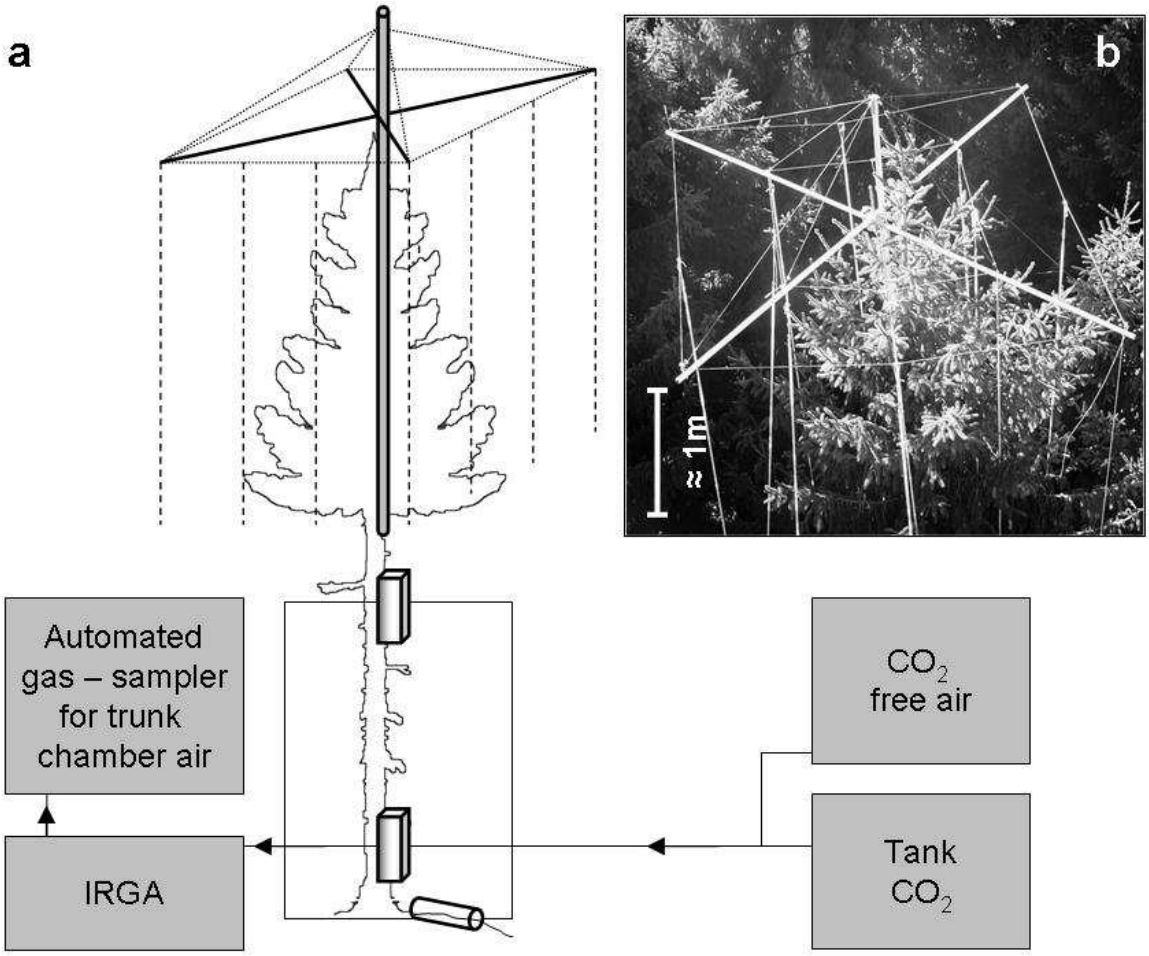


Fig. 3.1: (**a**) Flow-through system for the assessment of CO_2 efflux of upper and lower trunks and coarse roots, as well as the sampling of trunk chamber air for determination of $\delta^{13}C$. (**b**): Experimental setup of the isoFACE $^{13}CO_2/^{12}CO_2$ labeling infrastructure with micro porous CO_2-exposure tubes hanging through the canopy of adult spruce trees suspended from low-weight custom-build carrier structures.

Tree canopies were labeled throughout the daylight hours for 5 consecutive days. [CO_2] was monitored at two heights per tree (1 m and 3 m below the upper canopy edge) and above canopies. Membrane pumps continuously transported canopy air via PVC tubes to an IRGA (infra-red gas

analyzer, Binos 4b, Emerson Process Management, Weißling, Germany) and mass-flow of label into canopies was adjusted by these data. Canopy air at each sample position was collected once per day for the analysis of $\delta^{13}C_{Air}$. To this end, excess air from membrane pump outlets was flushed through 12ml Exetainer® vials (Labco Limited, High Wycombe, UK) using a 100mL syringe. Gas samples were analyzed for $\delta^{13}C$ by IRMS (isotope-ratio mass spectrometry, GVI-Isoprime, Elementar, Hanau, Germany). The $\delta^{13}C$ of labeled canopy air ($\delta^{13}C_{Air\text{-}Labeled}$) was calculated using data on canopy [CO_2] and linear regressions derived from Keeling plots of $\delta^{13}C_{Air}$ samples.

Three labeling experiments were conducted on the same trees of each species, i.e. in spring towards the end of leaf development, in early summer during times of highest trunk growth respiration rates (R_G), and in late summer under declining R_G. In beech, labeling started on May 24, July 9 and August 22, 2008, in spruce on June 23 and September 9, 2008, and on April 20, 2009. Comparison of $\delta^{13}C$ of CO_2 efflux of labeled trees with unlabeled control trees confirmed, that no C label from the previous experiments was found before a new 5-day labeling experiment was started.

Trunk and coarse root CO_2 efflux

Trunk and coarse root CO_2 efflux was assessed from day 0 through at least day 9 of the labeling experiment. Plexiglass® (Röhm GmbH, Darmstadt, Germany) chambers were laterally attached at two trunk heights (upper trunk: 9.5 ± 1.4 m, lower trunk: 0.7 ± 0.0 m), enclosing a trunk surface area of 203 to 534 cm². One coarse root per tree was completely enclosed in a cylindrical chamber. Throughout the measurements, all chambers were darkened with aluminized polyester foil. Each chamber was equipped with a temperature sensor (negative temperature coefficient, Pt 100, SEMI 833 ET, Hygrotec® Messtechnik GmbH, Titisee, Germany) to measure bark temperature (T_{Bark}).

CO_2 efflux was assessed by a computer-controlled flow-through gas-exchange system (Grams *et al.*, 2011; Kuptz *et al.*, 2011b). A stable reference gas (Fig. 3.1, 406.6 ± 0.9 µmol CO_2 mol^{-1}; $\delta^{13}C$ = - 3.06 ± 0.02 ‰, mean ± SE) was continuously pumped via PVC tubes through trunk and coarse root chambers at a rate of approx. 0.5 l min^{-1}. Outgoing chamber air was analyzed by an IRGA. Each chamber was sampled at about 3h intervals. Trunk CO_2 efflux was expressed per unit volume (m^{-3}) of living tissue behind the chamber (sapwood + bark, Maunoury *et al.*, 2007). Coarse root CO_2 efflux was related to the total root volume enclosed in chambers (Marsden *et al.*, 2008).

Growth respiration (R_G) was calculated for each sampling position using the mature-tissue method (Amthor, 1989; Kuptz *et al.*, 2011b). Accordingly, respiration rate (R) relates to bark temperature as given by Eqn. 3.1, assuming R to equal CO_2 efflux:

$$R = R_{15} * Q_{10}^{\frac{T_{Bark} - 10}{15}} \quad ,(\mu mol\ m^{-3}\ s^{-1}) \qquad \text{Eqn. (3.1).}$$

R_{15} represents the CO_2 efflux at 15 ℃ and Q_{10} the temperature sensitivity of the CO_2 efflux. For each sampling position, Q_{10} and R_{15} of maintenance respiration (R_M) were determined from measurements of CO_2 efflux and T_{Bark} during times without growth, i.e. from November 2008 to February 2009 (Kuptz *et al.*, 2011b). R_M during labeling experiments was then calculated using winter values for Q_{10} and R_{15} and actual T_{Bark} during the experiments. R_G was assessed as CO_2 efflux - R_M. We note that [CO_2] dissolved in the xylem sap may influence CO_2 efflux (Teskey *et al.*, 2008). However, during the measurement campaigns of this study, such an effect was negligibly small (Kuptz *et al.*, 2011b, see also discussion).

CO_2 sampling for stable carbon isotope analysis

To analyze the stable C isotope composition ($\delta^{13}C$) of CO_2 efflux ($\delta^{13}C_E$), chamber air and reference gas air were collected by means of an automated gas sampler (Gilson 221 XL, Gilson Inc. Middleton, USA), flushing air vented from the IRGA of the flow-though gas-exchange system (Fig. 3.1) through 12 ml glass vials (Exetainer®, Labco Limited, High Wycombe, UK). Trunk $\delta^{13}C_E$ was calculated using $\delta^{13}C$ and [CO_2] of chamber and reference air by applying a two-end-member mixing model (Grams *et al.*, 2011; Kuptz *et al.*, 2011b). Sampling interval was about 6h for each chamber.

The $\delta^{13}C_E$ released from coarse roots was analyzed twice a day (early morning and at the beginning of night) by means of a custom-build closed-respiration system (Grams *et al.*, 2011; Kuptz *et al.*, 2011b). Briefly, six sample vials were continuously flushed and isolated from the system at 10 min intervals. Coarse root $\delta^{13}C_E$ was calculated using the "Keeling Plot Approach" (Keeling, 1958; Zobitz *et al.*, 2008), rejecting Keeling plots with coefficients of determination (R^2) < 0.97.

The fraction of labeled carbon in CO_2 efflux (f_{Label}, see Lehmeier *et al.*, 2008; Gamnitzer *et al.*, 2009) was calculated for each sample position as:

$$f_{Label} = \frac{\delta^{13}C_{Old} - \delta^{13}C_{Sample}}{\delta^{13}C_{Old} - \delta^{13}C_{New}} \quad \text{Eqn. (3.2),}$$

where $\delta^{13}C_{Sample}$ represents the C isotope composition of the CO_2 efflux, and $\delta^{13}C_{Old}$ the $\delta^{13}C_E$ on day 0. $\delta^{13}C_{New}$ (i.e. fully labeled $\delta^{13}C_E$) was estimated for each sampling position and experiment individually by first calculating the apparent ^{13}C discrimination ($\Delta^{13}C_{A-E}$) between canopy air ($\delta^{13}C_{Air}$) and $\delta^{13}C_E$ on day 0:

$$\Delta^{13}C_{A-E} = \left(\frac{1000 + \delta^{13}C_{Air}}{1000 + \delta^{13}C_E} - 1\right) * 1000 \quad (‰), \quad \text{Eqn. (3.3).}$$

Then $\delta^{13}C_{New}$ was calculated as:

$$\delta^{13}C_{New} = 1000 * \frac{1000 + \delta^{13}C_{Air(labeled)}}{1000 + \Delta^{13}C_{A-E}} - 1000 \quad (‰), \quad \text{Eqn. (3.4).}$$

Before calculation of f_{Label}, all $\delta^{13}C_E$ samples of labeled trees were corrected for the natural day-by-day variation in $\Delta^{13}C_{A-E}$ observed at the unlabeled control trees.

Maximum f_{Label} in CO_2 efflux ($F_{Label-max}$) was calculated for each sample position and experiment by fitting f_{Label} through a sigmoid regression:

$$f_{Label} = \frac{F_{Label-max}}{1 + e^{-\frac{t - t_0}{b}}} \quad \text{Eqn. (3.5),}$$

with the fit parameter *b* giving the slope coefficient of the regression, *t* the time of measurement and t_0 the instant of the regression inflection point.

Carbon translocation rates (CTR in m h^{-1}) were assessed both in labeled and control trees. For control trees, we first calculated time lags between photosynthetic C fixation and respiratory loss. To this end, Pearson's correlations were determined for each tree and experiment between time-series of T_{Air} and trunk chamber $\delta^{13}C_E$. Therefore, data sets of T_{Air} were time shifted against $\delta^{13}C_E$ in intervals of

0.125 d covering a time span of 0 to 9 days. Time lags derived directly from the time delay of the correlation with the highest correlation coefficient (R) (Kuzyakov & Gavrichkova, 2010). Then, CTR was calculated as:

$$CTR = \frac{h_C - h_{TC}}{t_l} \quad (\text{m h}^{-1}), \qquad \text{Eqn. (3.6)},$$

whereby t_l represents the time lag (h), h_C the mean height of the tree canopy (m) and h_{TC} the height of the trunk chamber (m). For labeled trees, CTR was calculated in a similar way. First, time lags between f_{Label} of upper and lower trunks were assessed as described above. Then, CTR were calculated following Eqn. 3.6 using the distance between upper and lower trunk chambers.

Sampling of phloem sugars

On day 0 and day 5 of labeling, bark and phloem tissue material (∅ 5 mm, n = 3 each tree) were collected next to the lower trunk chambers. Phloem sap was extracted for 5 h at 4 ℃ in 15 mM sodium polyphosphate buffer (Sigma-Aldrich, Munich, Germany, Gessler *et al.*, 2004). Subsequently solution was centrifuged and phloem extract was analyzed for water soluble sugars (i.e. sucrose, fructose, glucose, raffinose and stachyose) by means of HPLC (high-pressure liquid chromatography, CARBOsep CHO-820 calcium column, Transgenomic, Glasgow, UK; for further details see Fleischmann *et al.*, 2009). Sucrose was partly broken down to glucose and fructose due to invertase activity in the sample solution. Hence, monosaccharides and disaccharides (i.e. sucrose, glucose and fructose) were integrated. Stable C isotope composition ($\delta^{13}C_P$) was assessed by IRMS attached to an element analyzer (EA3000, Euro Vector, Milan, Italy).

Statistical analyses

All statistical analyses were performed with PASW Statistics 18.0 (SPSS GmbH Software, Munich, Germany) and Sigma Plot 9.0 (Systat Software GmbH, Erkrath, Germany). Tests applied were Student's t-Test and Pearson's correlation as well as linear and sigmoid regression analysis.

Results

Weather conditions during labeling

Mean T_{Air} during the beech experiments was 16.5 ± 0.1, 15.8 ± 0.1 and 16.4 ± 0.1 ℃ in spring, early and late summer, respectively (Fig. 3.2). In the case of spruce, mean T_{Air} was 11.7 ± 0.1, 19.1 ± 0.1 and 9.9 ± 0.1 ℃, respectively (highest level d uring early summer, $p \leq 0.001$; Fig. 3.2). Short rainfall events (up to 13 mm per day) occurred during evenings of the labeling experiments in early summer (Fig. 3.2). Variation in vapor pressure deficit (VPD) followed the diurnal pattern in T_{Air} (data not shown).

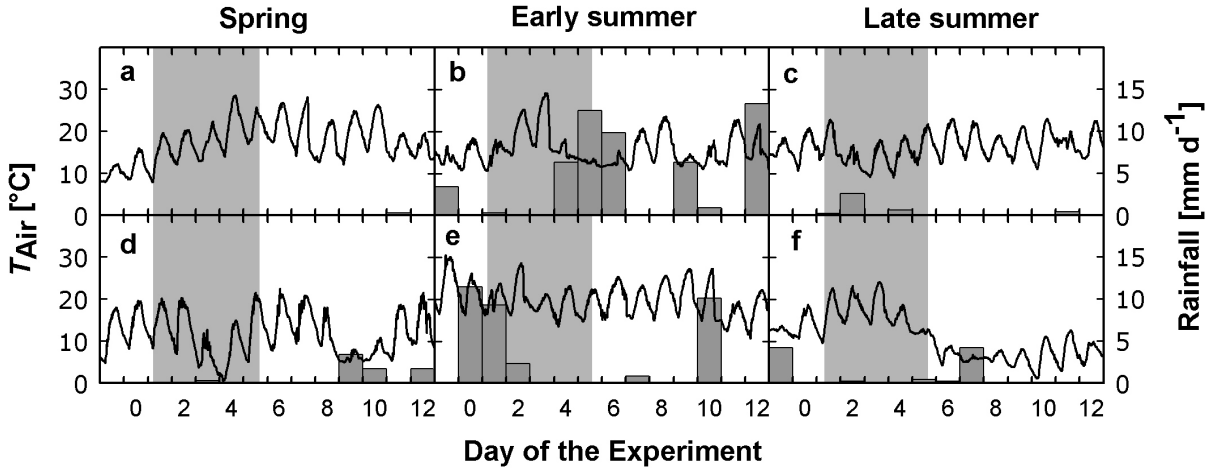


Fig. 3.2: Weather conditions for adult beech (**a - c**) and spruce (**d – f**) trees during the three labeling experiments. Air temperature (T_{Air}) is represented by the solid line, rain fall by gray columns. Gray areas give times of label application (day 1 – 5).

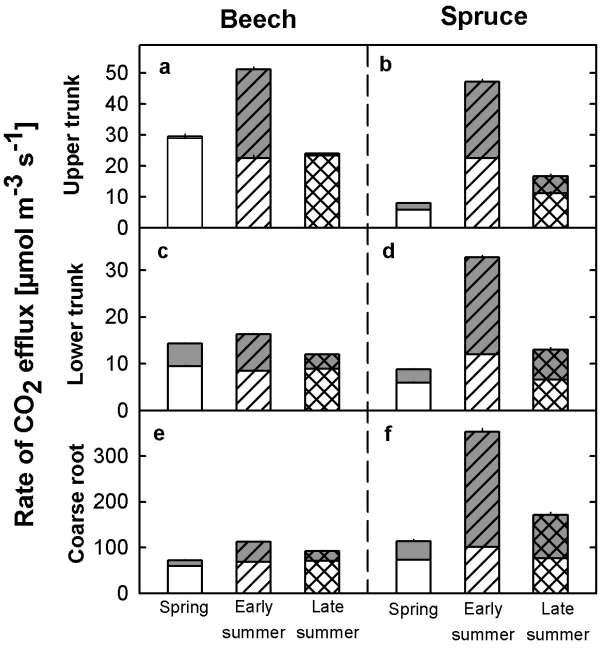


Fig. 3.3: Average rates of CO_2 efflux of upper (**a + b**) and lower tunks (**c + d**) and coarse roots (**e + f**) of adult beech and spruce trees during the three labeling experiments (spring, early summer, late summer). Rate of CO_2 efflux is represented by sum of gray and white bars, estimated maintenance respiration (R_M) by white bars. Data shown are means (± SE) of labeled trees (n = 4) during the experiments.

In both species, mean CO_2 efflux and R_G of trunk and coarse roots were highest during early summer and significantly lower in spring and late summer ($p \leq 0.001$, Fig. 3.3). Early summer experiments of both species were conducted near times when trunk CO_2 efflux was maximal (Fig. 3.3, see also Kuptz *et al.*, 2011b). CO_2 efflux was positively correlated with T_{Bark} during the preceding 3 to 9 hours, with Pearson's correlation coefficients (R) ranging between 0.78 – 0.99 ($p \leq 0.01$).

Stable C isotope label application

During label application, the increase in $[CO_2]$ in canopies was 123.5 ± 8.0 and 107.3 ± 6.8 µmol mol^{-1} for beech and spruce (means ± SE), respectively, on average over all experiments. $[CO_2]$ stayed for more than half of the exposure time within a ± 10% range of the target concentration of ambient $[CO_2]$ + 100 µmol mol^{-1} (Table 3.1). In the spring experiment of spruce and the late summer experiment of beech, CO_2 with a $\delta^{13}C_{Label}$ of about -36‰ was used (Table 3.1). For the spruce experiment in spring this resulted in a reduction of $\delta^{13}C_{Air}$ by 5.7‰. For all other experiment, the mean shift in $\delta^{13}C_{Air}$ during labeling was -8.6 ± 0.3‰.

Species	Experiment	Increase in $[CO_2]$	Time fraction within 10 % target $[CO_2]$	$\delta^{13}C_{Label}$	$\delta^{13}C_{Air}$	Shift in $\delta^{13}C_{Air}$ during labeling
Beech	Spring	110.4 ± 14.3	0.52	-46.3 ± 0.5	-7.1 ± 0.0	-8.9 ± 0.9
	Early summer	113.2 ± 13.2	0.64	-46.3 ± 0.8	-8.3 ± 0.0	-8.7 ± 0.7
	Late summer	146.9 ± 11.5	0.54	-36.9 ± 0.4	-8.7 ± 0.0	-7.4 ± 0.5
Spruce	Spring	94.2 ± 9.3	0.65	-35.2 ± 0.2	-7.5 ± 0.0	-5.7 ± 0.4
	Early summer	117.9 ± 15.2	0.46	-45.2 ± 0.6	-8.2 ± 0.0	-8.8 ± 0.9
	Late summer	109.7 ± 9.9	0.58	-44.6 ± 0.6	-8.0 ± 0.0	-9.2 ± 0.6

Table 3.1: Shifts in stable C isotope composition ($\delta^{13}C$) of canopy air due to labeling

Data shown are the increase in $[CO_2]$ during labeling (µmol mol^{-1}) and stable C isotope composition of label CO_2 ($\delta^{13}C_{Label}$) and of canopy air ($\delta^{13}C_{Air}$) (means ±SE).

Label recovery in $\delta^{13}C_E$ and $\delta^{13}C_P$

Over all experiments, the mean natural $\delta^{13}C_E$ of beech control trees was -27.6 ± 0.1, -28.3 ± 0.1 and -27.4 ± 0.1 ‰ in upper trunks, lower trunks and coarse roots, while the $\delta^{13}C_E$ of spruce controls was -27.3 ± 0.1, -27.1 ± 0.1 and -26.1 ± 0.2 ‰ (mean ± SE), respectively. Control tree $\delta^{13}C_E$

displayed distinct day-by-day variations of up to 3.4 and 3.3 ‰ in beech and spruce (Fig 3.4). In general, the $\delta^{13}C_E$ of labeled trees was not significantly different from the control trees at day 0, but declined 2 to 4 days after the reduction of $\delta^{13}C$ in canopy air (Fig. 3.4).

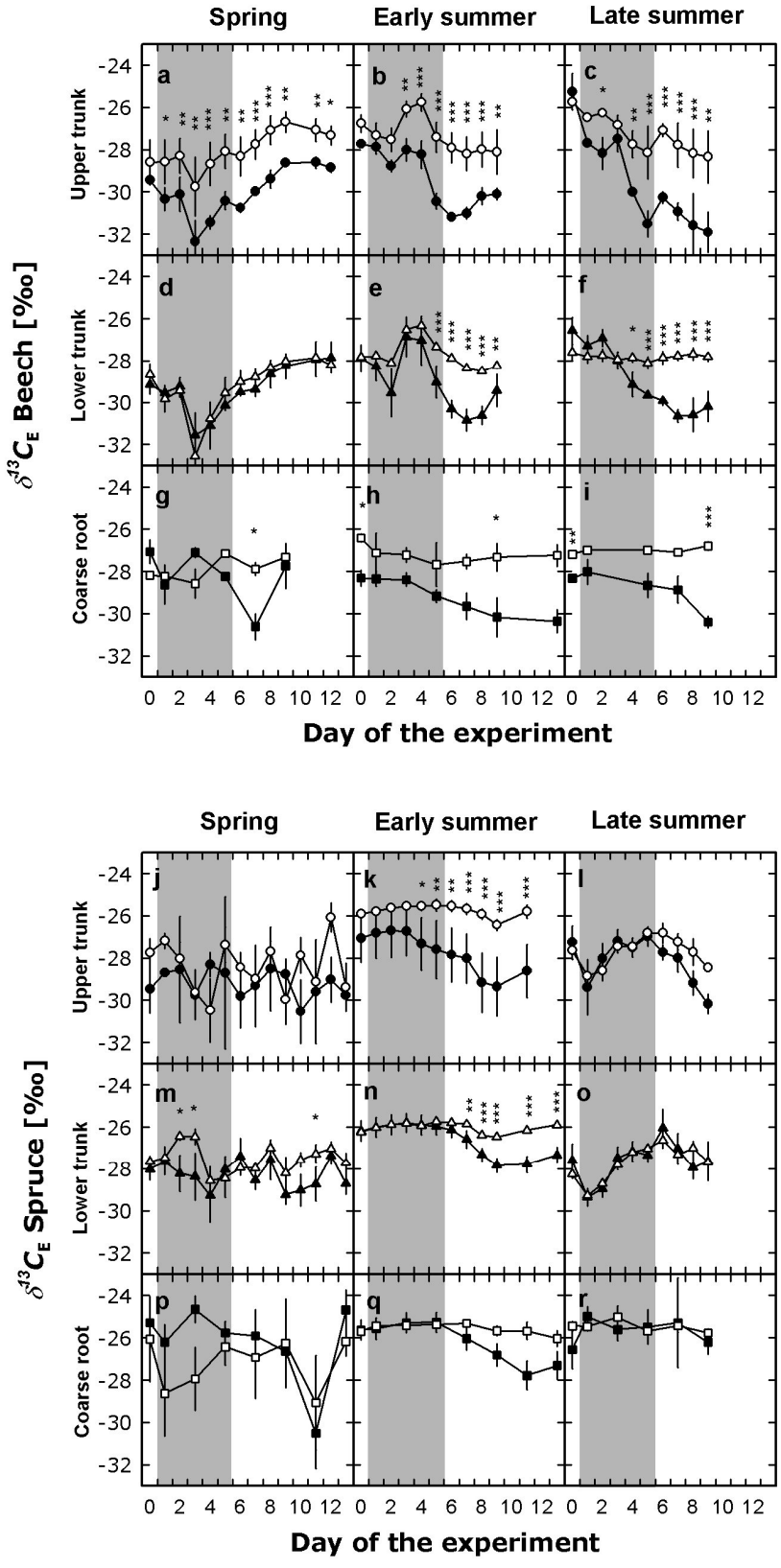


Fig. 3.4: Stable C isotopic composition of CO_2 efflux ($\delta^{13}C_E$) during the labeling experiments of beech (**a - i**) and spruce (**j - r**) sampled from upper (circles) and lower trunks (triangles) and coarse roots (squares). Data shown are daily means (± SE). Closed symbols give labeled trees, open symbols control tress. Significant differences between labeled and control trees of $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$ are represented as *, ** and ***, respectively. Gray areas give times of label application (day 1 – 5).

In spring, this decline in $\delta^{13}C_E$ of labeled beech trees was not observed for lower trunks and, with the exception of one data point, for coarse roots. During summer, $\delta^{13}C_E$ of coarse roots in controls was initially somewhat higher than in the labeled trees, nevertheless the latter displayed the declining pattern 7 to 9 days after the start of label application. Compared to day 0 and control trees, trunk $\delta^{13}C_E$ of labeled beech trees declined strongest during late summer (about 4.0 ‰). In spruce, the decline in $\delta^{13}C_E$ of labeled trees was consistently most pronounced during early summer (-2.1, -1.7 and -2.2 in upper trunks, lower trunks and coarse roots, respectively). Conversely, hardly any change in response to label application was observed during spring and late summer. The total concentration of phloem sugars in beech (sum of sucrose, fructose, glucose, raffinose and stachyose) did not vary seasonally (13.5 ± 0.9, 12.4 ± 0.7 and 12.1 ± 0.9 mg g^{-1} dry matter). Conversely in spruce, significantly higher sugar concentrations, in particular of oligosaccharides were recorded in spring compared to summer (94.7 ± 5.5, 20.7 ± 2.0 and 14.1 ± 0.2, mean ± SE, $p \leq 0.001$, Fig. 3.5). During the early summer experiment of spruce, $\delta^{13}C_P$ was about 2 ‰ lower than the corresponding $\delta^{13}C_E$ at the lower trunk position. In all other experiments, linear regressions between $\delta^{13}C_E$ and $\delta^{13}C_P$ resembled the 1:1 relationship (Fig. 3.5).

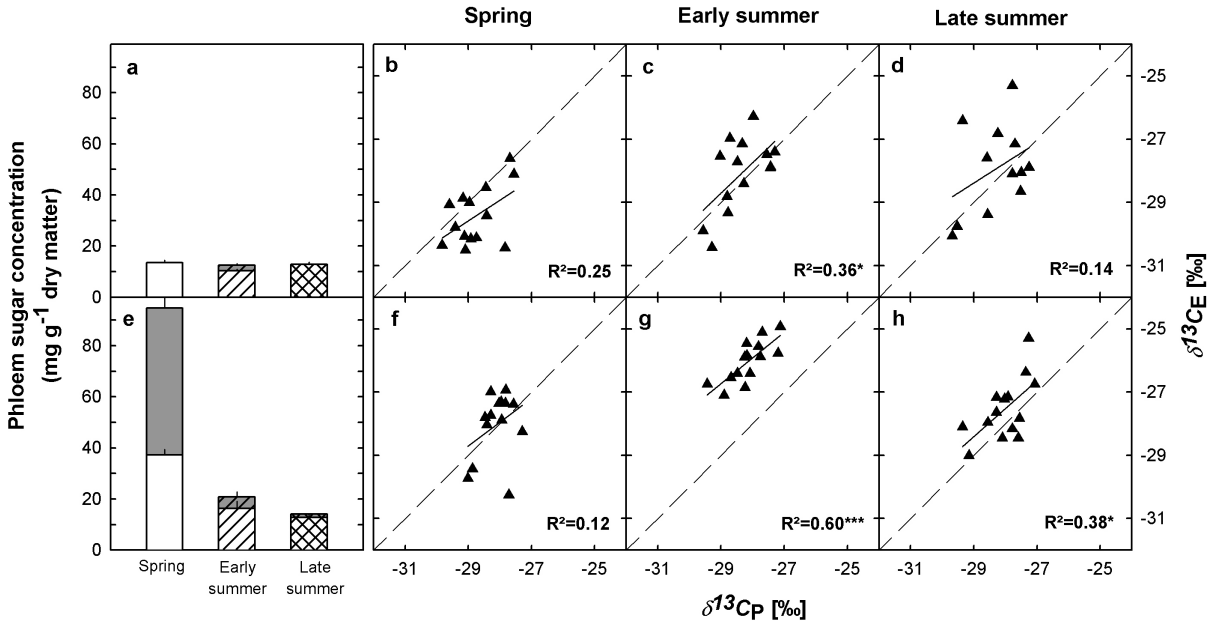


Fig. 3.5: Concentration of phloem sugars (white = sum of sucrose, fructose and glucose, gray = sum of other sugars, mainly raffinose and stachyose) in mg g^{-1} dry matter of beech (**a**) and spruce (**e**) and linear regressions between the stable C isotope composition of phloem sugars ($\delta^{13}C_P$) and lower trunk CO_2 efflux ($\delta^{13}C_E$) during spring (**b + f**), early summer (**c + g**) and late summer (**d + h**) of both labeled and control trees. Continuous line gives regression, dashed line the 1:1 relationship. Significance levels at p ≤ 0.05 and p ≤ 0.001 are represented as * and ***, respectively.

In beech, the fraction of labeled C (f_{Label}) in trunk or coarse root CO_2 efflux was generally small during spring and consistently increased only at the upper trunk position (up to 0.23; Fig. 3.6a). Maximum in f_{Label} was recorded in coarse roots during early summer (0.4 ± 0.2) and in trunks during late summer (upper trunks: 0.6 ± 0.2, lower trunk: 0.5 ± 0.1; Fig. 3.6c, f).

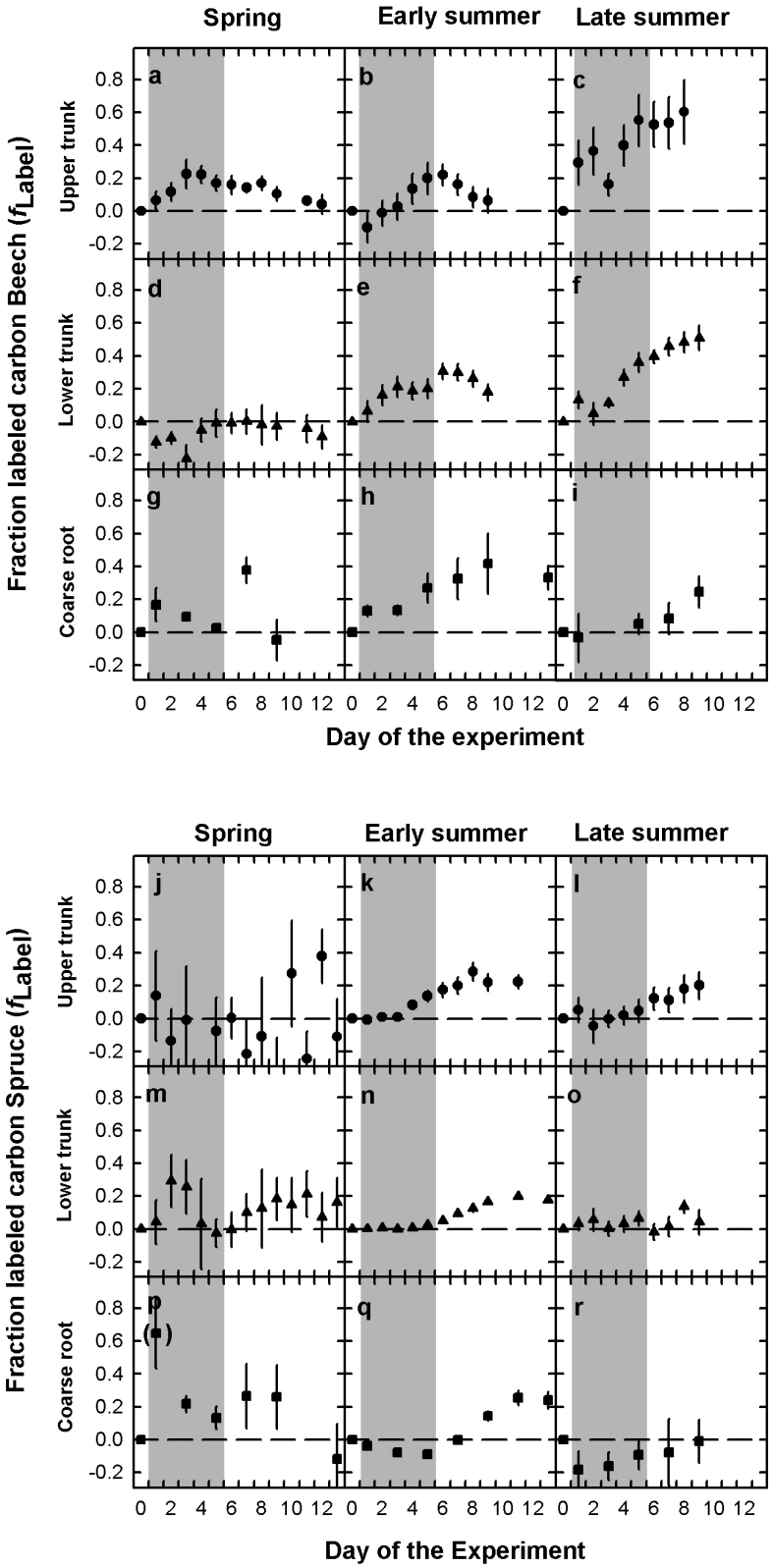


Fig. 3.6: Fraction of labeled carbon (f_{Label}) in CO_2 efflux of upper (circles) and lower trunks (triangles) and coarse roots (squares) during labeling experiments of beech (**a - i**) and spruce (**j - r**). Data shown are daily means (± SE). Gray areas give times of label application (day 1 – 5).

During the early summer experiment, f_{Label} peaked first in upper trunks, followed by lower trunks and coarse roots. In trunks, f_{Label} declined 2 to 3 days after the end of the label application, which was not the case in late summer when f_{Label} stayed for at least for 4 days after the end of label application at higher values. In spruce, pronounced changes in f_{Label} were observed only during the early summer experiment and, in the case of upper trunks also during late summer (Fig 3.6b). Spruce f_{Label} did not decline within a couple of days after the end of label application during early summer as observed in beech. Similar to beech, f_{Label} peaked first in upper then in lower trunks and finally in coarse roots (Fig. 3.6).

In beech, fitted maxima of f_{Label} ($F_{Label\text{-}Max}$) of upper and lower trunks were low in spring (0.2 ± 0.1 and 0.1 ± 0.1, respectively) and maximal in late summer (upper trunks: 0.6 ± 0.3, lower trunks: 0.6 ± 0.1, see Fig. 3.7). Conversely, $F_{Label\text{-}Max}$ of beech coarse root was lowest in late summer (0.1 ± 0.1) and maximal in early summer (0.4 ± 0.2). In spruce, no significant difference in $F_{Label\text{-}Max}$ was observed throughout the year and always stayed below 0.3. In contrast to $F_{Label\text{-}Max}$, seasonal dynamics in the contribution of R_G to CO_2 efflux were uniform in both species and always maximal during early summer (beech: 0.4 - 0.6, spruce: 0.5 – 0.8, Fig. 3.7). High contribution of R_G was paralleled by high CO_2 efflux rates (see bar width in Fig. 3.7 c, d).

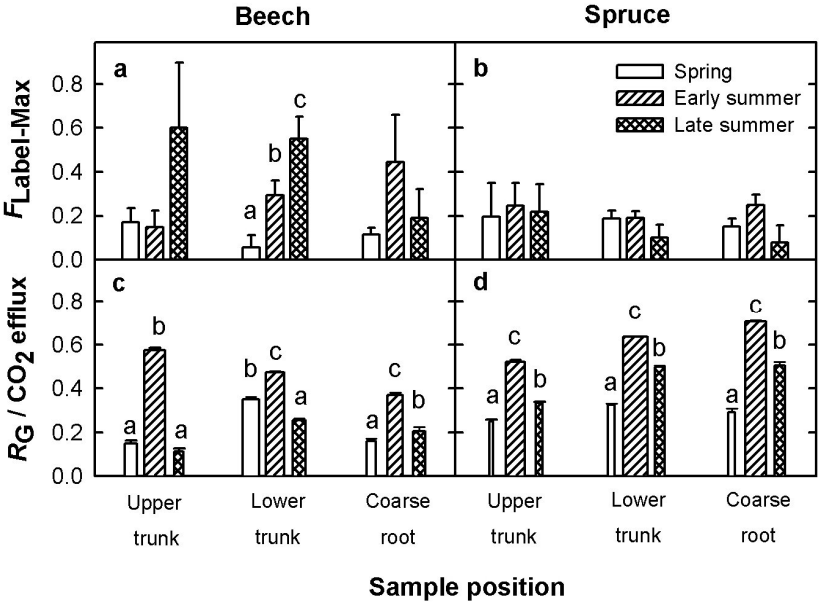


Fig. 3.7: Mean fractional contribution of labeled C to CO_2 efflux ($F_{Label\text{-}Max}$) of beech (**a**) and spruce (**b**) trees and the quotient of the rate of growth respiration (R_G) to CO_2 efflux for beech (**c**) and spruce (**d**) during the labeling experiments. Bar width in **c** and **d** give relative CO_2 efflux rate of organs between experiments. Lower case letters give significant differences at the same sampling position between experiments with $p \leq 0.05$.

Carbon translocation rates

Carbon translocation rates (CTR) in trunks of labeled trees were similar to rates assessed in control trees (Fig. 3.8). Rates ranged between 0.15 – 0.72 m h^{-1} and between 0.06 – 0.44 m h^{-1} for beech and spruce, respectively. Species displayed distinct seasonal differences with higher CTR in early summer compared to spring and late summer. However, this was only significant in control trees ($p \leq 0.05$). At each time point, spruce transport rates were 50 % or less than those in beech.

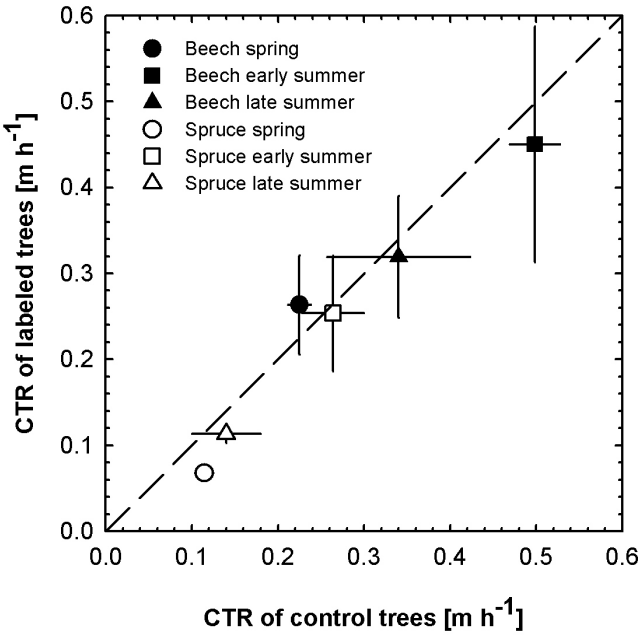


Fig. 3.8: Carbon translocation rates (CTR) of control and labeled trees in the three labeling experiments of beech (closed symbols) and spruce (open symbols). Data shown are means (± SE).

Discussion

Whole canopies of adult beech and spruce trees were labeled with stable C isotopes during spring, early and late summer, i.e. at the end of leaf development, maximal rates of trunk growth respiration (R_G) and at declining R_G, respectively. Distinct seasonal dynamics in allocation of recent photosynthates to woody tissue CO_2 efflux were recorded at two trunk and one coarse root position, both in magnitude and time point of label appearance.

In both species, CO_2 efflux and R_G displayed distinct seasonal patterns in trunks and coarse roots (Fig. 3.3). Both parameters and the contribution of R_G to CO_2 efflux (Fig. 3.7) were highest during early summer, suggesting high activity of growth processes such as diameter growth or lignin

synthesis (Stockfors & Linder, 1998). In beech, second highest levels were found in spring (lower trunks) and late summer (coarse roots, Fig. 3.7), indicating that trunk growth had already started in spring (Maunoury *et al.*, 2007), followed by root growth late in the growing season (Smith & Paul, 1988). In spruce, second highest contributions of R_G to CO_2 efflux were consistently found during late summer, indicating high activity of growth processes late in the season (Kuptz *et al.*, 2011b).

As recently discussed in the literature, CO_2 dissolved in the xylem sap might influence woody tissue CO_2 efflux (Teskey *et al.*, 2008). In our experiments, regression slopes between $\delta^{13}C_E$ and $\delta^{13}C_P$ were close to the slope of the 1:1 relationship (Fig. 3.5), indicating that a shift in $\delta^{13}C_P$ results in the same shift in $\delta^{13}C_E$ without perceivable contribution of sap flow dissolved CO_2. We therefore conclude that sap flow had no or only a small effect on $\delta^{13}C_E$ during our experiments, which is in line with recent findings (Ubierna *et al.*, 2009; Grams *et al.*, 2011; Kuptz *et al.*, 2011b). During early summer, $\delta^{13}C_E$ of spruce was nearly 2 ‰ enriched in ^{13}C compared to $\delta^{13}C_P$ (Fig. 3.5). This offset may be related to intensive lignin synthesis. Since lignin is known to be depleted in ^{13}C compared to most respiratory substrates, CO_2 respired during its synthesis has to be enriched (Hobbie & Werner, 2004; Bowling *et al.*, 2008). A similar trend can be seen during the early summer experiment of beech, however not as pronounced as in spruce (Fig. 3.5).

Distinct seasonal dynamics in f_{Label} and $F_{Label\text{-}Max}$ were observed in beech. In spring, only small amounts of label were found at all three sampling positions, although high rates of R_G indicated active growth in lower trunks during this time. The highest amounts of label were recovered in late summer, when R_G already declined (Fig. 3.7). The observed seasonal patterns may be interpreted by the involvement of two distinct C pools in beech trunks, a transfer and a storage pool (Fig. 3.9). In spring, recent photosynthates only marginally supplied R_G of beech (Fig. 3.7). At this time, foliage and shoot development may be the strongest sinks for recent photosynthates (Hansen & Beck, 1994; Dickson *et al.*, 2000) and both R_G and R_M of trunks appear to be supplied by C stores accumulated during the previous years (Fig. 3.9, Helle & Schleser, 2004; Kagawa *et al.*, 2006; Skomarkova *et al.*, 2006). In early summer, C allocation to beech trunks increased with the demand for growth (Mordacq *et al.*, 1986; Hansen & Beck, 1994). During this time, f_{Label} in trunks declined shortly after the end of label application, suggesting high turn-over of recent photosynthates in C pools involved in trunk respiration (Fig. 3.6). Thus, labeled C may not have entered the storage pool, which typically has higher half-lives (Ritter *et al.*, 2010), and was either respired directly (i.e. R_G) or allocated to below-ground sinks (Fig.

3.7, Hansen & Beck, 1990). In contrast, during late summer, f_{Label} of trunks reached highest levels and did not decline within a couple of days after the end of label application.

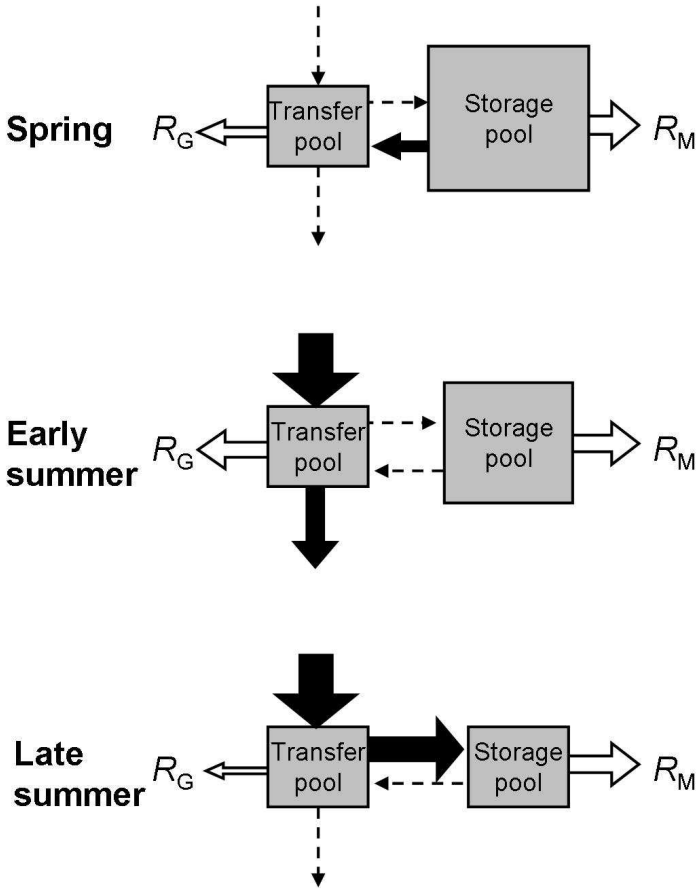


Fig. 3.9: Conceptual two-pool model (one transfer and one storage C pool) of the substrate supply for lower trunk CO_2 efflux of adult beech trees during spring, early and late summer. R_G and R_M are growth and maintenance respiration, respectively. During spring, negligible amounts of new photosynthates enter the transfer pool and both R_G and R_M are predominately supplied by stored C. During early summer, new photosynthates enter the transfer pool, directly supplying R_G and transported to coarse roots. R_M is still supplied by stored C. During late summer, new photosynthates enter both the transfer and storage pool, supplying both R_G and R_M. Size of open arrows relate to mean flux size of lower trunk R_G and R_M during labeling experiments of beech in comparison to the annual maxima of mean daily CO_2 efflux recorded from May 2008 till June 2009.

The longer label retention time suggests slower label turn-over and involvement of the storage pool (Fig. 3.6, 3.9). Thus, labeled C may also be incorporated into the storage pool, since demand for R_G was already declining (Fig. 3.9). During late summer, trunks are known to be strong C sinks due to the accumulation of C for winter storage and frost hardiness (Hansen & Beck, 1990; Skomarkova *et al.*, 2006). Thus, $F_{Label\text{-}Max}$ peaked during late summer in trunks, since both R_G and R_M are supplied with labeled C (Fig. 3.9). As a consequence, the size of the storage pool is assumed to be shrinking during spring and early summer and to be refilled during late summer. Accordingly, Barbaroux & Breda (2002) reported a distinct decline of sugar concentration in the youngest 10 tree rings of beech

between May and July, followed by an increase later in the season. Assuming the same decline in sap wood sugar concentration (about 5 mg g^{-1} dry mass) in our study trees, this would result in an estimated C loss of 3.0 ± 0.3 g m^{-3} sapwood. This is about half of the C released by R_M from May to July, indicating that R_M can easily be supplied by stored C during this time period. Overall, during spring, both R_G and R_M of beech were largely supplied by C stores. Thus, our first hypothesis that R_G is exclusively supplied by recent photosynthates was rejected for this time of the year. During late summer, hypothesis two that R_M is supplied by storage C was not supported, as both R_G and R_M were predominantly supplied by recent photosynthates. However, during the main growing period, i.e. early summer, both hypotheses are supported by our results.

In spruce, $F_{Label\text{-}Max}$ hardly changed throughout the year (Fig. 3.7). As an evergreen species, spruce displayed less dependence on C stores because C reserves may be refilled by periods of active photosynthesis during mild winter days (Hansen *et al.*, 1996; Hu *et al.*, 2010; Kuptz *et al.*, 2011b). Overall, smaller amounts of label were recovered in spruce and f_{Label} did not decrease after the end of label application. Both observations indicate that recent photosynthates consistently mix with the C stores before they are respired. Thus, R_G is not directly and exclusively supplied by recent photosynthates. During spring, high phloem sugar concentrations were observed, in particular oligosaccharides such as raffinose and stachyose (Fig. 3.5), while during summer, phloem sugars consisted mainly of disaccharides (sucrose). We assumed that, in spruce, raffinose and stachyose were mainly formed from storage C and we concluded that respiration during spring was partially supplied by C reserves. Overall, it is unlikely that R_G in spruce was exclusively supplied by recent photosynthates, so that for spruce, hypothesis (I) was rejected. Conversely, hypothesis (II) claiming R_M to be supplied by storage pool C was supported by our results.

The interpretation of our data with a two-pool model is supported by the transport rates of phloem sugars. Overall, carbon translocation rates (CTR) of 0.06 to 0.72 m h^{-1} were consistent with previous studies (Keitel *et al.*, 2003; Mencuccini & Hölttä, 2010; Wingate *et al.*, 2010). CTR calculated from time lags between the natural variation in $\delta^{13}C_E$ and T_{Air} gave similar transport rates as CTR calculated from labeling, giving support for the comparability of labeling and natural abundance approaches in determining C transfer along trees (Kuzyakov & Gavrichkova, 2010; Mencuccini & Höttä, 2010; Wingate *et al.*, 2010). C transport in spruce was consistently slower compared to that in beech, which may reflect anatomical differences, e.g. sieve cells in gymnosperms versus highly specialized sieve tube systems in angiosperms (Schulz & Thompson, 2010; Wingate *et al.*, 2010). In

both species, CTR was highest during early summer (Fig. 3.8). Phloem sap velocity may be sensitive to temperature (Johnsen *et al.*, 2007), but this cannot explain the seasonal pattern of beech, because T_{Air} was lowest in early summer. The seasonal dynamics may rather relate to enhanced C sink strength of the growing plant tissue in early summer (Kozlowski, 1992; Wingate *et al.*, 2010).

Conclusion

Our study demonstrated pronounced seasonal dynamics in C transport rate and the contribution of recent photosynthates to the CO_2 efflux of adult beech and spruce trees. High CO_2 efflux and C transport rates during early summer were related to intense growth metabolism during this time. Thereby, C transport rates along beech and spruce trunks that were calculated by time lags between natural $\delta^{13}C_E$ variation and T_{Air} provided similar results to transport rates calculated from C isotope labeling experiments. Seasonal dynamics in the contribution of recent photosynthates to respiration were interpreted in relation to a two-pool model, consisting of transfer and storage C. Hypothesis (I) that growth respiration was exclusively and directly supplied by recent photosynthates was only supported for beech during early summer. Hypothesis (II) claiming maintenance respiration to be supplied by C that previously passed through a storage pool was largely supported. We conclude that the contribution of recent photosynthates and C stores to respiration is highly variable in deciduous beech, as recent photosynthates were turned over faster during early summer and slower in late summer due to allocation to C stores. Evergreen spruce, in contrast, is deemed to be less dependent on seasonal build-up and consumption of stored C, so that the contribution of recent photosynthates to respiration was rather uniform throughout the growing season. The observed seasonal dynamics in C transport and use of photosynthates for respiration and storage should be considered for modeling of ecosystem carbon balances.

4. General Discussion

The present thesis focused on seasonal dynamics determining the CO_2 efflux of non-leafy plant organs of adult European beech and Norway spruce trees. Thereby, two experimental approaches were used (chapter 2 & 3). First, the natural variation in $\delta^{13}C_E$ of the CO_2 efflux was quantified over the course of a whole year (chapter 2). Second, three stable C isotope labeling experiments were performed per species at different phenological stages by labeling the pool of recent photosynthates of whole-tree canopies (chapter 3). The following chapter (chapter 4) will present a synopsis of both studies by discussing the experimental design, by elaborating consistencies in the results from both approaches and by providing novel evidence derived from their combination.

Methodological application

Both experimental systems described in the present study, the isoFACE infrastructure and the flow-through gas-exchange chamber system, proofed to be suitable designs to investigate C dynamics in tree CO_2 efflux (Grams *et al.*, 2011; Kuptz *et al*., 2011a; Kuptz *et al.*, 2011b). Chambers have been extensively used to measure both CO_2 efflux rate and $\delta^{13}C_E$ of branches, trunks and roots (Damesin & Lelarge, 2003; Maunoury *et al.*, 2007; Acosta *et al.*, 2008). These measurements were mainly done by connecting individual chambers to a portable IRGA or collecting chamber air for IRMS analysis by a syringe. Such sampling techniques require manual handling and are time consuming. In the present study, such tasks were carried out automatically (chapter 2, 3, appendix A). Moreover, the presented design allows for the use of new, commercially available TDL laser spectrometry providing online sampling of $\delta^{13}C$, prolonged measurement campaigns and high sampling frequency (Bowling *et al.*, 2003; Wingate *et al.*, 2010).

Whole tree canopies were successfully labeled using the isoFACE infrastructure (chapter 3, appendix A). Concerns might occur on the transferability of the isoFACE design to other field sites, since it might require carrier structures for tube suspension, i.e. a stationary scaffolding (Werner & Fabian, 2002). The measurements on spruce (chapter 3, Kuptz *et al.*, 2011a), however, demonstrated that stationary scaffoldings are, in fact, not needed, since exposure tubes can be suspended by low-weight carrier structures which are attached to the main tree axis. Carrier structures can easily be installed at monopodial growing trees, i.e. Norway spruce, but their installation might be challenging at

monochasial trees like European beech. A canopy crane has proven useful for their installation (Basset *et al.*, 2003).

The isoFACE infrastructure was originally designed for steady-state labeling of recent photosynthates in adult trees (appendix A, Grams *et al.*, 2011). The present study restricted labeling to 5 consecutive days (chapter 3). Thus, experiments presented in this study were intermediates between recently developed pulse labeling studies (Högberg *et al.*, 2008; Plain *et al.*, 2009) and long-term steady-state labeling experiments (Schnyder *et al.*, 2003; Lehmeier *et al.*, 2008). Both pulse and long-term steady-state labeling approaches were considered unsuitable in view of the study aims of this thesis. Pulse labeling requires a strong label, i.e. pure ^{13}C or ^{14}C (Carbone *et al.*, 2007; Högberg *et al.*, 2008) and remaining label might be recovered in CO_2 efflux or bulk tissues for up to 4 months (Keel *et al.*, 2007; Plain *et al.*, 2009). Long-term steady-state labeling may cause large quantities of label to be incorporated into slowly turned-over storage pools (Lehmeier *et al.*, 2008). Such long label retention times were neither desired in view of the seasonal investigation on natural $\delta^{13}C_E$ variations done at the same trees (chapter 2, Kuptz *et al.*, 2011b), nor during repeated label application within one growing season (chapter 3, Kuptz *et al.*, 2011a). Therefore, the present study used 'extended' pulses of low label intensity. As a result, label did not emerge from the CO_2 efflux for more than one month. The present study demonstrated that both long-term steady-state labeling (appendix A) and "extended" pulse labeling experiments (chapter 3) are accomplishable using the isoFACE infrastructure.

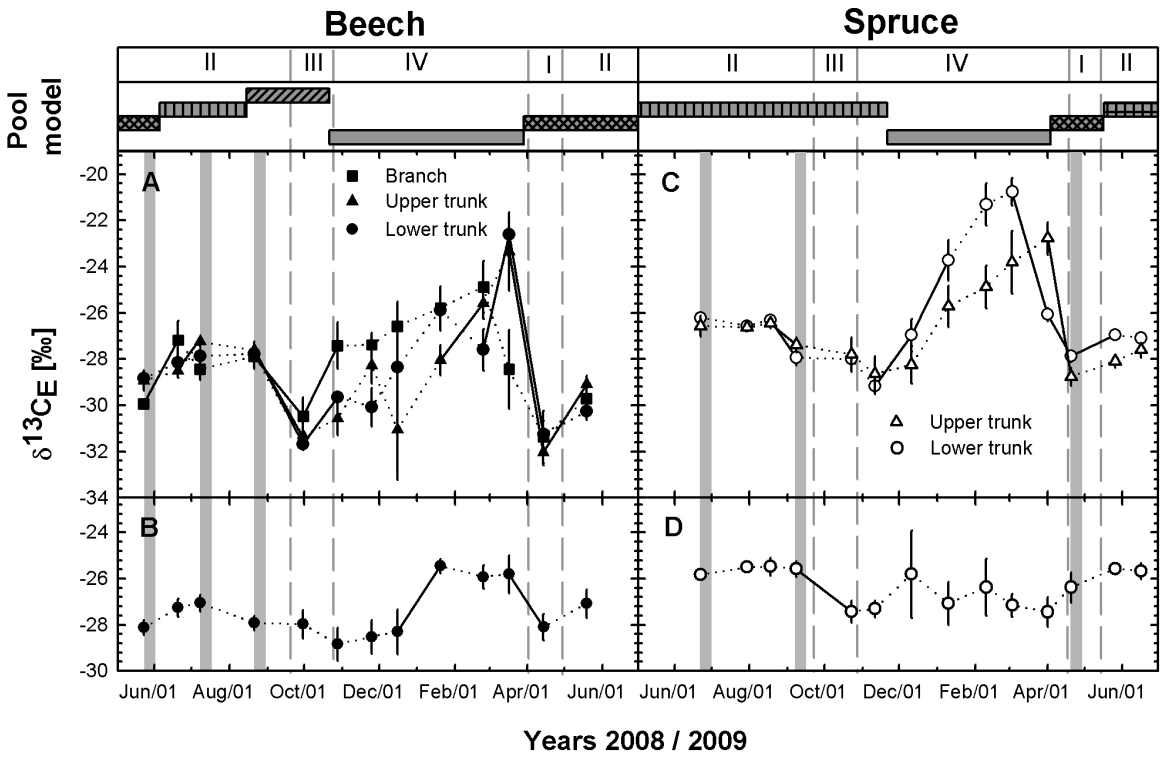
Combination of natural isotopic abundance and labeling

Both the natural isotopic abundance approach and experimentally applied isotopic labels are frequently used tools in plant research. For instance, natural abundance experiments provide insight into plant respiratory processes (Tcherkez *et al.*, 2003; Bathellier *et al.*, 2009). Moreover, temporal variations in natural $\delta^{13}C_E$ might result from changes in photosynthetic fractionation (Brandes *et al.*, 2006). Such "natural tracers" may serve for quantifying C transport velocities in plants (Kuptz *et al.*, 2011a; Kuzyakov & Gavrichkova, 2010; Wingate *et al.*, 2010). As natural tracer experiments might be restricted under constant meteorological conditions (Kodama *et al.*, 2008; Kodama *et al.*, 2010), labeling of recent photosynthates may provide more reliable information on C transport velocities at any time. In addition, labeling experiments allow for more precise C flux partitioning (Lehmeier *et al.*, 2008; Plain *et al.*, 2009). In the present study, a combination of both "natural $\delta^{13}C$" and "labeling"

approaches was used to analyze $\delta^{13}C_E$ and the CO_2 efflux rate of adult forest trees. As the findings of both experiments were already discussed in chapter 2 and 3, the following section will elucidate consistencies and highlight novel evidence derived from the combination of both approaches.

CO_2 dissolved in the xylem sap might influence both CO_2 efflux rate (Teskey *et al.*, 2008; Hölttä & Kolari, 2009) and its $\delta^{13}C$ signature (Moore *et al.*, 2008). Throughout the present study, however, no influence of sap flow on $\delta^{13}C_E$ and only a slight interference with the CO_2 efflux rate were observed (Tab. 2.4, Fig. 3.5 and Fig. appendix A.5). A large proportion of the CO_2 dissolved in the xylem sap is considered to originate from autotrophic respiration (Teskey *et al.*, 2008). Thus, the $\delta^{13}C$ of autotrophic respiration and xylem dissolved CO_2 should closely resemble each other, complicating their isotopic distinction based on the natural abundance of ^{13}C. However, since the label-induced shift in $\delta^{13}C_E$ consistently equaled the shift in $\delta^{13}C_P$ of phloem sugars (Fig. 3.5, Fig. appendix A.5), the present study led to the conclusion that CO_2 emitted by woody tissue of adult forest trees mainly consisted of autotrophic respiration (Wertin & Teskey, 2008; Maier *et al.*, 2009). Overall, it was concluded that sap flow had no distinguishable influence on woody tissue $\delta^{13}C_E$, as previously observed by Ubierna *et al.* (2009) in large conifer trees.

Combining the results from both experimental approaches (Kuptz *et al.*, 2011a; Kuptz *et al.*, 2011b) on the seasonal dynamics underlying branch, trunk and coarse root CO_2 efflux provided a coherent picture (Fig. 4.1). Thereby, results from chapter 3, i.e. C pool models developed for interpreting the labeling data, fitted results from chapter 2, i.e. seasonal dynamics in the natural $\delta^{13}C_E$. For each pool model of chapter 3, a time period was assigned along the seasonal scale (Fig. 4.1). During leaf flush, foliage and shoot development was considered as the strongest C sink in both species (period I, Hansen *et al.*, 1996). Only small amounts of label were found in trunk and coarse root CO_2 efflux (Fig. 3.7), while natural $\delta^{13}C_E$ values during period I closely resembled those during leaf senescence (period III, Fig. 4.1). Thus, during spring, both the "natural $\delta^{13}C_E$" and the "labeling" approach coincide in that branch, trunk and coarse root CO_2 efflux of both species was mainly supplied by storage C that was accumulated during previous years (see Fig. 4.1 model a, Kagawa *et al.*, 2006). Moreover, spring labeling of beech indicated that pool model (a) was still valid until the end of May. During this time, shoot elongation and shoot diameter growth might still be a strong C sink for recent photosynthates (Hansen *et al.*, 1996).



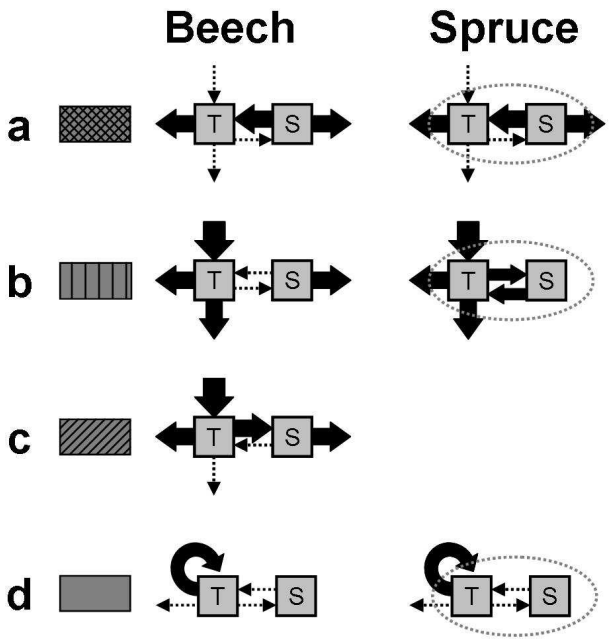


Fig. 4.1: Modification of Figure 2.6 "Annual changes in $\delta^{13}C$ of CO_2 efflux ($\delta^{13}C_E$) of branches, trunks and coarse roots". Graphs in **A + C** give $\delta^{13}C_E$ of non-leafy branches and of upper and lower trunks, graphs in **B + D** of coarse roots. Time points of labeling experiments are highlighted by vertical gray bars. Individual C pool models a - d for beech and spruce are given by the legend and represented by different patterns (horizontal bars). Thereby, T represents a transfer, S a storage pool. Gray ellipses in pool models a, b and d of spruce symbolize the close association of the transfer and the storage pool with each other and that the respiratory supply system of spruce might also be explained by one instead of two C pools.

During early summer, labeling of beech indicated growth respiration to be the strongest C sink for recent photosynthates. These findings coincide with increased lignin synthesis presumed by the ^{13}C enriched natural $\delta^{13}C_E$. Lignin is ^{13}C depleted to most respiratory substrates by 5 - 6 ‰ (Bowling *et al.*, 2008) and the remaining respiratory substrate during its synthesis might be relatively ^{13}C enriched (Fig. 4.1 model b, Hobbie & Werner, 2004; Ocheltree & Marshall, 2004). The late summer labeling of beech suggested active storage accumulation already before leaf senescence (Skomarkova *et al.*,

2006), while the drop of the natural $\delta^{13}C_E$ in period (III) indicated increased starch synthesis during senescence. Thus, beech storage accumulation might have lasted from shortly before the beginning of senescence until leaf fall (Fig. 4.1 model c).

While the presented pool models (a – c) matched the natural $\delta^{13}C_E$ dynamics in beech CO_2 efflux from leaf flush to leaf senescence (period I to III), the continuous $\delta^{13}C_E$ increase observed during winter dormancy (period IV), which was also seen in spruce, could not be explained by either model. The best explanation was malate accumulation through PEPc activity (Fig. 4.1 model d, see also chapter 2, Gessler *et al.*, 2009b). During this time, the contribution of stored C was regarded to be rather small, as concentrations of respiratory substrates in phloem and woody tissue are usually reported to stay stable during winter (Höll, 2000; Barbaroux & Breda, 2002).

Compared to deciduous beech, evergreen spruce depended less on the seasonal accumulation and consumption of C stores (chapter 2 & 3, Cerasoli *et al.*, 2004; Trumbore, 2006; Hu *et al.*, 2010). Instead, spruce labeling suggested a rather constant mixing of old and new respiratory substrates throughout most of the year and a more uniform contribution of both recent photosynthates and C stores to autotrophic respiration (chapter 3, Fig. 4.1 model b). Likewise, seasonal variations in the natural $\delta^{13}C_E$ dynamics were less pronounced in spruce than in beech (chapter 2). Ritter *et al.* (2010) proposed that the respiratory supply system of spruce saplings might be best explained by a one-pool model instead of two C pools. The authors argued that angiosperm trunks possess higher abundances of living parenchyma cells in the secondary phloem and xylem than gymnosperms. In beech, such parenchyma cells might serve as a storage pool, spatially separated from the phloem tissue (Höll, 2000). Such a storage pool might be lacking in spruce. Indeed, in the case of spruce, both transfer and storage pool C seemed to be more closely associated with each other than in beech, but a distinct conclusion of whether the respiratory supply system of spruce is better related to one rather than two C pools was not possible (Fig. 4.1, model a, b and d). Nonetheless, regardless of the number of associated C pools, the contribution of old respiratory substrate to autotrophic respiration in adult spruce was evident. In contrast to beech, it was assumed that C stores in spruce most likely serve as a short-term buffer to meet the continuous C demand of autotrophic respiration (Lehmeier *et al.*, 2010) rather than to supply trees over long periods without access to recent photosynthates, i.e. as in deciduous trees during winter dormancy.

Concluding remarks

Overall, the combination of both approaches, based on “natural” and “labeled” isotopic pattern, provided novel and consistent evidence about seasonal changes of beech and spruce CO_2 efflux. The present study highlighted differences between plant organs (branches, trunks and coarse roots) and contrasting foliation (deciduous vs. evergreen). The observed seasonal patterns were largely related to plant phenology, i.e. changes in respiratory metabolic activity (chapter 2), changes in C allocation and in the contribution of different C pools to respiration (chapter 3). The presented pool models highlighted the importance of storage build-up and consumption in beech, while a clear distinction of a transfer and a storage pool was less pronounced in evergreen spruce. Time periods of transition from one pool model to another, as they were presented in Fig. 4.1, however, should not be considered definite, as these were estimated from the data sets. In fact, defining time points of seasonal transition from one model to another might be challenging, as transition might occur either gradually (i.e. smoothly declining sink strength of growth respiration) or abrupt (i.e. TCA prevailing PEPc refixation at rising temperatures in spring). Although the presented results are consistent between beech and spruce, they may not to be valid for other species. The influence of plant phenology on CO_2 efflux may differ among species and sites (Trumbore, 2006) and may also be largely affected by climate change (Hughes, 2000; Walther *et al.*, 2002).

Trumbore (2006) stated that to date, a “theory of respiration” is missing, given the large knowledge gaps on autotrophic and heterotrophic respiration. In this respect, the present study strengthened the mechanistic understanding on autotrophic respiration of adult forest trees, as it demonstrated that tree phenology largely affected woody tissue CO_2 efflux of adult European beech and, to a lesser extent, of Norway spruce. Such influences have to be considered aiming towards comprehensive understanding of forest ecosystem respiration.

Appendix A: A free-air system for long-term stable carbon isotope labeling of adult forest trees[3]

Summary

Stable carbon (C) isotopes, in particular employed in labeling experiments, are an ideal tool to broaden our understanding of C dynamics in trees and forest ecosystems. Here, we present a free-air exposure system, named isoFACE, designed for long-term stable C isotope labeling in the canopy of 25 m tall forest trees. Labeling of canopy air was achieved by continuous release of CO_2 with a $\delta^{13}C$ of -46.9‰. To this end, micro-porous tubes were suspended at c. 1 m distance vertically through the canopy, minimizing CO_2 gradients from the exterior to the interior and allowing for C labeling exposure during periods of low wind speed. Target for CO_2 concentration ($[CO_2]$) increase was ambient + 100 µmol mol^{-1}. Canopy $[CO_2]$ stayed within 10% of the target during more than 57% of the time and resulted in a drop of $\delta^{13}C$ in canopy air by 7.8‰. After 19 labeling days about 50% of C in phloem sugars and stem CO_2 efflux were turned over and 20-30% in coarse root CO_2 efflux and soil CO_2. The isoFACE system successfully altered $\delta^{13}C$ of canopy air for studying turn-over of C pools in forest trees and soils, highlighting their slow turn-over rates.

[3] Published as Grams TEE, Werner H, Kuptz D, Ritter W, Fleischmann F. Andersen CP, Matyssek R. (2010), *Trees – Structure and Function*, DOI: 10.1007/s00468-010-0497-7

Introduction

Carbon (C) allocation of trees plays a critical role in C cycling in forest ecosystems (Litton *et al.*, 2007). Respiratory C flux from trees to the atmosphere is a major component of the C budget of forests (Luyssaert *et al.*, 2010). In total, roughly half of the C fixed by forest trees in the temperate climate zone is released back to the atmosphere as respiratory CO_2 within a couple of days. A large part of this respiratory C flux can be attributed to whole-tree (up to 50%, Ryan, 1991; Paembonan *et al.*, 1992) and stem respiration (10 to 22%, Acosta *et al.*, 2008; Tang *et al.*, 2008; Wieser *et al.*, 2009). However, our knowledge about allocation of recently fixed C to the various sites of respiration within a forest ecosystem (e.g. leaf, branch, stem, root, soil) is still limited. In spite of the magnitude and importance of this respiratory C flux in the global C budget (IPCC, 2007a), its response to global change is not well understood (Moore *et al.*, 2008).

Carbon isotopes are ideal tools for studying the C fluxes through plants and ecosystems. Earlier labeling experiments using ^{14}C have been largely replaced (but not completely, see e.g. Leake *et al.*, 2001; Joslin *et al.*, 2006; Sauer *et al.*, 2006; Carbone *et al.*, 2007) by the application of stable C isotopes (i.e. ^{12}C and ^{13}C). The change has been motivated by the easier handling and lower analytical costs as well as the lower discrimination of ^{13}C compared to ^{14}C in metabolic pathways and diffusion (Ehleringer, 1991). The recent availability of commercial laser spectrometer for the analysis of stable C isotope composition ($\delta^{13}C$) in CO_2 will intensify the use of stable carbon isotopes as tracers, in particular when investigating respiratory C fluxes (e.g. Plain *et al.*, 2009; Wingate *et al.*, 2010). Those investigations will broaden our understanding of the isotopic composition of leaf, branch, stem and, in particular, root and soil respiration (Bowling *et al.*, 2008).

In spite of its ideal nature for C labeling experiments, limitations inhere to stable C isotope labeling techniques, constraining their use to certain environments or plant types. For instance, application of C labeling to model systems cultivated in growth chambers proved to be enormously useful for quantifying C flux rates and pool sizes (Dyckmans & Flessa, 2001; Schnyder *et al.*, 2003; Lattanzi *et al.*, 2005; Lehmeier *et al.*, 2008). However in the field, C label application as CO_2 is mostly limited to smaller plants such as grasses, herbs or juvenile trees (Simard *et al.*, 1997; Ostle *et al.*, 2007; Talhelm *et al.*, 2007). Similar labeling studies on forests are scarce and often took advantage of free-air concentration enrichment (FACE) experiments that investigate tree responses to long-term increased [CO_2] (e.g. Keel *et al.*, 2006; Moore *et al.*, 2008). Those approaches, however, focus on trees that have been adapted to growth under an increased CO_2 atmosphere.

More recently, new C labeling systems have been developed for forest trees either enclosing groups of 4-m-tall boreal *Pinus sylvestris* trees or canopies of individual trees by means of plastic covers (Högberg *et al.*, 2008; Plain *et al.*, 2009; Subke *et al.*, 2009). Hence, the atmosphere in closed systems needs to be cooled to keep air temperatures close to ambient, and trees are labeled by insertion of highly concentrated $^{13}CO_2$ resulting in up to 5.7 atom% ^{13}C inside the plastic covers (Högberg *et al.*, 2008). However, environmental parameters such as [CO_2] and vapor-pressure deficit of the atmosphere (VPD) may not be controlled in a satisfactory way, potentially affecting C allocation patterns. Nevertheless, those kinds of C labeling systems allow for application of high amounts of ^{13}C tracer and represent a powerful tool for studying timing and dynamics of C fluxes (Högberg *et al.*, 2008; Plain *et al.*, 2009).

While the above mentioned systems represent a large progress in C allocation studies of forest tress, there are still limitations in the size and duration of the label application. The aim of the present effort is to develop a C labeling system that permits long-term labeling of adult forest trees under free air and, hence, virtually unchanged environmental conditions to study e.g. C allocation to respiratory C pools in trees. Here we present the design of such a system and data from a series of pilot experiments. Focus is on system performance in view of C label application within the canopy of adult forest trees including data highlighting changes in respiratory C pools to demonstrate the label incorporation by the trees.

Material and Methods

Site description

The study was carried out in a mixed beech/spruce stand in southern Germany ("Kranzberg Forest", elevation 485 m a.s.l., 48°25'N, 11°39'E; for details see Pretzsch *et al.*, 1998) on adult European beech trees (*Fagus sylvatica* [L.]) about 60 to 70 years old and up to 25m high. Long-term mean annual air temperature and rainfall (1970-2000) were 7.8 °C and 786 mm, respectively (Matyssek *et al.*, 2007). The site is characterized by low wind velocities with above-canopy annual means <1.7 m s^{-1} (Heerdt, 2007). Inside-canopy wind velocity was estimated to be reduced by 80% relative to the above-canopy reference position (Winterhalter, 1998; Nunn *et al.*, 2005). Scaffolding and a 45 m stationary research crane, equipped with a 50 m boom and a working gondola allowed for access to the canopy of the trees and facilitated the experimental set-up.

Experiments

In the following we report on four stable C isotope labeling experiments which were conducted (1) to test for the efficacy of label application within the canopy of adult beech trees and (2) to verify label uptake and use in respiratory pathways of trees and soils. Experiments #1 and #2 during Sept 2007 and May 2008, respectively, explicitly monitored the distribution of the applied CO_2 within the canopy of the trees. Experiments #3 and #4 conducted in August 2006 and July 2008, respectively, studied the effect of label application on physiological aspects of adult beech trees.

System assembly

The labeling infrastructure, named isoFACE, is a free-air exposure system designed to isotopically label complete crowns of adult forest trees to CO_2 of experimentally changed $^{13}CO_2/^{12}CO_2$ ratio under otherwise unchanged climatic conditions. The general design of the system resembles free-air concentration enrichment (FACE) systems which apply pure CO_2 released from pipes (Miglietta *et al.*, 2001; Okada *et al.*, 2001) or tubes (e.g. webFACE, Pepin & Korner, 2002) and an earlier C labeling system (Talhelm *et al.*, 2007). Deviating from those approaches, we used vertically hanging PVC tubes in the tree crowns (Fig. A.1A), similar to the O_3-FACE system used earlier at the same site (Werner & Fabian, 2002; Karnosky *et al.*, 2007). Suspension of tubes was realized about 2 m above the canopy attaching eight metal poles horizontally to cross at one central tower. This set-up allowed for spinning a metal rope resulting in a spiderweb-like carrier structure (Fig. A.1B). Subsequently, each of four adjacent beech crowns was supplied – depending on its size – with ten to twenty 30 m long vertically hanging fiber strengthened PVC tubes (Øout/in 15/9 mm), some of them placed about 1 m into the canopies of the surrounding trees. To ensure a homogeneous release of CO_2 into the canopy, each tube was micro-perforated by 400 glass capillaries (Øout/in 360/75 µm) at a distance of 2 cm each across the upper eight meters of the tube (Fig. A.1C). Per tree, total material costs for tubing, suspension and connection to the gas tank amount to about 1000€, excluding the cost for scaffolding or towers as supportive structure (see also discussion). Time for preparation (e.g. insertion on glass capillaries) and hanging of tubes by means of a canopy crane are estimated as 10 h per tree.

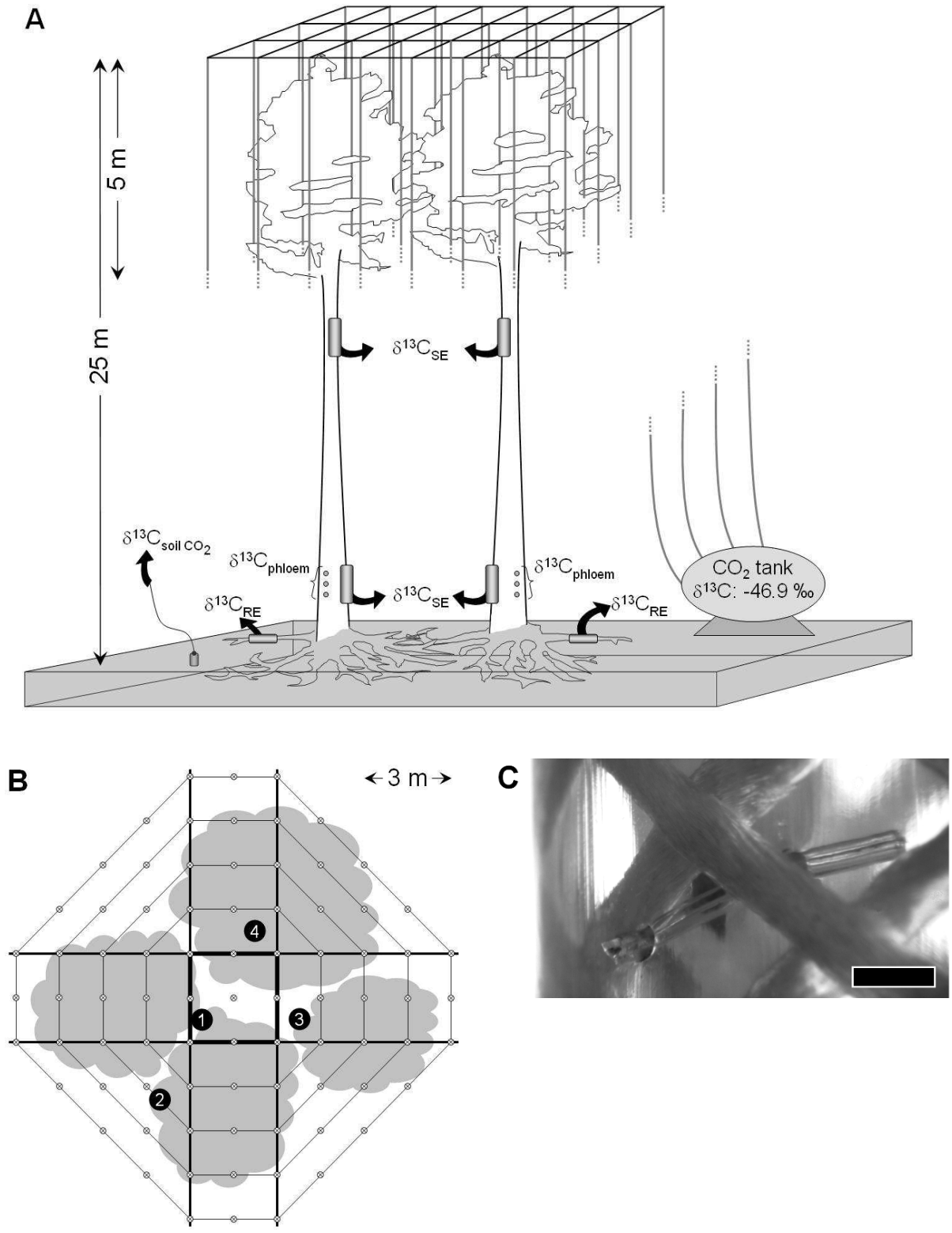


Fig. A. 1: (**A**) Schematic diagram of the isoFACE system with CO_2-exposure tubes hanging vertically through the canopy of adult beech trees. Coarse root chambers and stem chambers at both breast height and at the crown base were employed to sample CO_2 efflux from roots and stems for stable C isotope analysis ($\delta^{13}C_{RE}$ and $\delta^{13}C_{SE}$, respectively). Sampling for stable C isotope analysis of phloem sap was done next to the stem chambers at breast height and of soil gas at a distance of 0.2 to 0.5 m from the bole base of the tree ($\delta^{13}C_{phloem}$ and $\delta^{13}C_{soil\ CO2}$, respectively) (**B**) Top view with approx. positions of micro-porous PVC tubes. Numbers 1 to 4 give sampling positions for canopy air at three heights. (**C**) Glass capillary (Øout/in 360/75 µm) inserted in fiber strengthened PVC tubes. Black scale bar equals 1mm.

The stable C isotope labeling was conducted by addition of pure CO_2 with reduced $^{13}CO_2$ content (i.e. $\delta^{13}C$ of about -46.9‰; see below for definition of δ-values). The unavoidable consequence of this approach, increased CO_2 concentration ($[CO_2]$), was on average limited to 100 µmol CO_2 per mol total gas (hereafter refereed to as µmol mol^{-1}) and thus the $[CO_2]$ increase remained within the natural variation in temperate forests (Berry *et al.*, 1997; Mortazavi & Chanton, 2002). The CO_2 used for labeling was stored at the site in a tank with a capacity either for 1100 or 10000 kg CO_2. Typically 50-100 kg CO_2 per tree and day were used.

Canopy CO_2 concentration

CO_2 concentration within the labeling plot was monitored at four positions (see number 1 to 4 in Fig. A.1B). At each position canopy air was sampled at three heights: (i) at the canopy surface, (ii) 3m and (iii) 5m within the canopy, corresponding to the position of the sun, intermediate and shade crown, respectively. At the same respective heights, $[CO_2]$ was monitored in an unlabeled reference canopy. In addition, $[CO_2]$ was recorded at 5 m above the canopy and 2 m above the forest floor - twice within the labeling plot and once at the reference plot. At each sampling position a membrane pump was continuously sucking air through an about 30 m long PVC tube with a flow rate of about 1.4 L min^{-1}. Subsequently, a programmable multiplex system connected the sampled gas to an infra-red gas analyzer for $[CO_2]$ assessment (IRGA, Binos 4b, Emerson Process Management, Weißling, Germany). Each channel was analyzed about once per hour for three min and the average of four readings at 15 s intervals during the third minute was recorded.

The $[CO_2]$ above the canopy at the reference plot + 100 µmol mol^{-1} was used as the target concentration for the labeling plot. Flux of CO_2 from the tank through the exposure tubes was feedback controlled via a mass flow controller in response to the average $[CO_2]$ at the 12 probing positions (i.e. four positions at three canopy heights).

On May 27, 2008, $[CO_2]$ at canopy surface was monitored at higher temporal resolution (10 s intervals, experiment #2), employing four IRGAs of two Licor 6400 (Licor Inc., Lincoln, Nebraska, USA).

Mass spectrometry

All gas samples were analyzed within 48 hours on an isotope ratio mass spectrometer (IRMS, GVI-Isoprime, Elementar, Hanau, Germany) interfaced to a gas autosampler (Gilson 221 XL, Gilson

Inc. Middleton, USA). Carbon isotope ratios ($\delta^{13}C$) are expressed in δ-notation using Vienna PeeDee Belemnite (VPDB) as the standard. The $\delta^{13}C$ is calculated as $(R_{Sample}/R_{VPDB} - 1) * 1000$, where R_{sample} and R_{VPDB} represent the isotopic ratios of $^{13}C/^{12}C$ of the sample and VPDB, respectively. Repeated measurements of the same gas showed a precision of the IRMS of $\delta^{13}C < 0.15‰$ (SD; $n = 10$).

Monitoring $\delta^{13}C$ of atmospheric CO_2

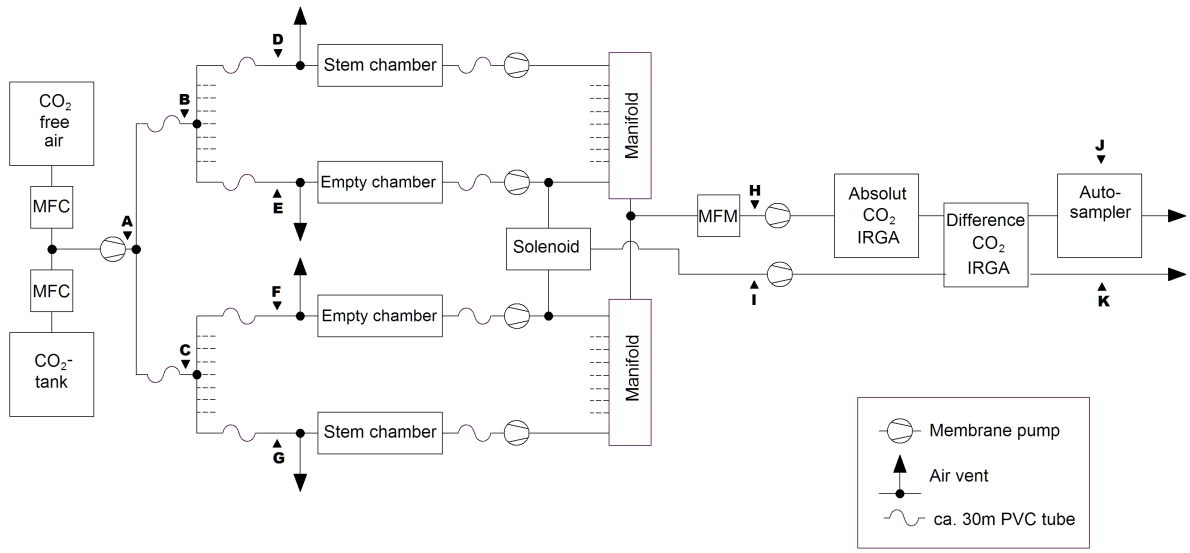
Air from all sampling positions was sampled daily (between 10:00 and 13:00 h) from the gas outlet behind the membrane pumps. To this end, a 10 cm long needle was attached to a gas-tight 100 mL syringe. Care was taken to fully insert the needle into the gas outlet and to slowly (< 10 mL s^{-1}) sucking the gas into the syringe. Applying a second needle, the sample volume was flushed through an Exetainer® vial (volume of 12 mL, Labco Limited, High Wycombe, UK) and sampled gas was analyzed by IRMS.

Stem CO_2 efflux

Stem CO_2 efflux was assessed at a lower (breast height) and an upper stem position (15 to 17 m) of each study tree. Each chamber was attached to the stem at a position previously cleaned from mosses, lichens and algae and sealed to the bark with rubber sealant (Terostat IX, Henkel, Düsseldorf, Germany) and acrylate (Lugato, Hamburg, Germany). Chambers were leak-tested by applying a slight over-pressure (ca. 2000 Pa) while tolerating a pressure drop of < 100 Pa min^{-1} and darkened with aluminized polyester foil.

Sampling of CO_2 efflux from stems was automated by a computer-controlled open gas exchange system that comprised 20 channels, including two reference gas channels (see schematic diagram in Fig. A.2). The air streams through the 20 channels were standardized by processing, as a first step, ambient air with a molecular sieve (Zander KEN 3100, Essen, Germany) for drying and removal of CO_2. Subsequently, constant $[CO_2]$ of 400 µmol mol^{-1} was established by adding CO_2 from a gas cylinder by means of a mass flow controller, and air was re-humidified to a dew point of 5-10 ℃ depending on air temperature. The incoming, standardized air was split then into the 20 air streams which were pushed through c. 30 m long PVC tubes to 20 Plexiglas® (Röhm GmbH, Darmstadt, Germany) stem chambers (including two empty reference chambers, Fig. A.2). Chamber air was continuously sampled by a suite of membrane pumps. Surplus air was exhausted in front of chambers to minimize pressure increase. Absolute $[CO_2]$ and difference in $[CO_2]$ between empty and stem

chambers were assessed by two infra-red gas analyzers (IRGA, Binos 4b, Emerson Process Management, Weißling, Germany). Subsequently, sample air was flushed through Exetainer© vials by means of a double-hole needle inserted through the septum by an automated gas sampler (Gilson 221 XL, Gilson Inc. Middleton, USA). Vials were flushed for six minutes each, at a flow rate of at least 0.15 L min^{-1}. The sampling device, which can be equipped with a total of 220 vials, allowed for continuous sampling throughout almost 30 h. The various components of the system had been checked for isotopic neutrality along the gas path of the system by taking samples using a 100 mL syringe at 11 positions as marked in Fig. A.2 by letters "A" to "K". Maximum deviation in $\delta^{13}C$ by 0.15‰ from the reference air sampled at the different positions reflected the precision of the IRMS.



Position	A	B	C	D	E	F	G	H	I	J	K
	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)
Jul 17	-2.9 ± 0.1	-2.8 ± 0.0	-2.7 ± 0.2	-2.7 ± 0.1	-2.7 ± 0.1	-2.7 ± 0.1	-2.7 ± 0.1	-2.8 ± 0.1	-3.0 ± 0.1	-2.9 ± 0.1	-2.8 ± 0.1
Aug 14	-2.2 ± 0.1	-2.2 ± 0.1	-2.2 ± 0.1	-2.3 ± 0.1	-2.5 ± 0.1	-2.3 ± 0.1	-2.5 ± 0.1	-2.4 ± 0.1	-2.4 ± 0.1	-2.3 ± 0.1	-2.4 ± 0.1
Sep 2	-3.2 ± 0.1	-3.0 ± 0.1	-3.1 ± 0.1	-3.0 ± 0.1	-3.0 ± 0.0	-3.0 ± 0.1	-3.0 ± 0.1	-3.1 ± 0.1	-3.0 ± 0.1	-3.1 ± 0.1	-3.0 ± 0.0

Fig. A. 2: Schematic diagram of the open flow system for sampling of CO_2 from stems. Air of constant [CO_2] and $\delta^{13}C$ was pumped through PVC tubes to stem and empty reference chambers. A suite of membrane pumps continuously sampled chamber air for infrared gas analysis (IRGA) to assess absolute [CO_2] and the difference between empty and stem chambers. Subsequently, gas was sampled by programmable autosampler in 12 ml Exetainer© vials, which were flushed for 6 min at a flow rate of at least 0.15 L min^{-1}. The CO_2 sampling system was tested for stability in $\delta^{13}C$ of the gas three times during the growing season. To this end, samples were taken by means of a gas-tight 100 mL syringe at the marked positions ("A" to "K"). No significant change in $\delta^{13}C$ was detected along the system. MFC: mass flow controller, MFM: mass flow meter.

The $\delta^{13}C$ of CO_2 deriving from stem efflux ($\delta^{13}C_{SE}$ in ‰) was calculated using a two end-member mixing model (Dawson *et al.*, 2002) as follows:

$$\delta^{13}C_{SE} = \frac{\left([CO_2]_{sample} * \delta^{13}C_{sample}\right) - \left([CO_2]_{reference} * \delta^{13}C_{reference}\right)}{[CO_2]_{sample} - [CO_2]_{reference}} \quad (‰), \qquad \text{Eqn. A.1}$$

where

$[CO_2]_{sample}$ = CO_2 concentration of sample gas from a stem chamber (µmol mol^{-1}),

$[CO_2]_{reference}$ = CO_2 concentration of reference gas from an empty chamber (µmol mol^{-1}),

$\delta^{13}C_{sample}$ = $\delta^{13}C$ signature of sample gas from a stem chamber (‰) and

$\delta^{13}C_{reference}$ = $\delta^{13}C$ signature of reference gas from an empty chamber (‰).

Carbon isotope composition ($\delta^{13}C$) of coarse root CO_2 efflux

CO_2 from coarse root efflux was sampled around midday (10:00 and 14:00 h) by means of a custom-build closed respiration system (cf. Prater *et al.*, 2006; Kuptz *et al.*, 2011b). Individual root chambers were connected by means of decabon tubing (ZTR-Rossmanek GmbH, Balve, Germany) to a manifold system with six parallel sampling channels, each flushing a 12 mL Exetainer© vial. Prior to sampling, the system was flushed for 10 minutes with ambient air at a flow rate of about 0.5 L min^{-1}. For sampling, the system was operated in a closed mode for about 60 minutes, resulting in an increase of $[CO_2]$ inside the system caused by the CO_2 release from the coarse root. About every 10 minutes one Exetainer© vial was isolated by simultaneously closing solenoids directly in front and behind the vial. To obtain reliable estimates of $\delta^{13}C$ of CO_2 derived from coarse root efflux ($\delta^{13}C_{RE}$), we adjusted the sampling interval to attain a $[CO_2]$ range of ≥ 100 µmol mol^{-1} between the first and last sample. $\delta^{13}C_{RE}$ was calculated from the six gas samples taken at each measurement according to the "Keeling Plot approach" (Zobitz *et al.*, 2008). Keeling plots with coefficients of determination (R^2) < 0.97 were rejected.

Sampling of phloem sugars

Phloem sap was sampled from the lower stem position nearby the stem chamber following the procedure of Gessler et al. (2004) (Fig. A.1A). Small pieces of bark with adherent phloem tissue (∅ 5 mm) were cored from the stem and incubated (5 h at 4 ℃) in 15 mM sodium polyphosphate buffer

(Sigma-Aldrich, Munich, Germany). After centrifugation, a subsample of the phloem sap extract was transferred into tin caps, freeze-dried and analyzed in combined element analyzer (EA3000, Euro Vector, Milan, Italy) and IRMS for their C content (C_{sample}) and stable carbon isotope composition ($\delta^{13}C_{sample}$), respectively. A further subsample was analyzed by means of HPLC (CARBOsep CHO-820 calcium column, Transgenomic, Glasgow, UK) to quantify water soluble sugars (sum of sucrose, fructose and glucose, C_{PS}) in the phloem sap extract. Subsequently, $\delta^{13}C$ of sugar C in the phloem sap ($\delta^{13}C_{PS}$) was calculated as follows:

$$\delta^{13}C_{PS} = \frac{\left(C_{sample} * \delta^{13}C_{sample}\right) - \left(C_{NPS} * \delta^{13}C_{NPS}\right)}{C_{PS}} \quad (‰), \qquad \text{Eqn. A.2}$$

where

$\delta^{13}C_{NPS}$ = $\delta^{13}C$ of non-sugar C in phloem that was assumed not to be labeled during the experiment and therefore to equal $\delta^{13}C_{sample}$ before labeling (day 0), and

C_{NPS} = content of non-sugar C in phloem after labeling, calculated as the difference between C_{sample} and C_{PS}.

Assessment of soil respired CO_2

Soil gas samples were taken as described in detail by Andersen *et al.* (2010). In brief, specialized soil-gas sampling wells were buried around each tree at a distance of 0.2 to 0.5 m from the bole base at 8 cm depth (Fig. A.1A). Teflon tubing allowed for sampling 5-8 mL of soil gas from each buried sampler using a gas-tight syringe. Gas samples were subsequently transferred into 12 mL Exetainer® vials and samples were analyzed by IRMS as described below. $\delta^{13}C$ of soil respired CO_2 was calculated from $\delta^{13}C$ and $[CO_2]$ of soil gas samples and ambient air above the soil surface using a two-pool mixing model (Dawson *et al.*, 2002) as described in Eqn. A.1, whereby the reference gas was substituted with the ambient air above the soil surface. Please note that $\delta^{13}C$ of soil respired CO_2 was not adjusted by -4.4‰ to account for the more rapid diffusion of ^{12}C compared to ^{13}C (Andersen *et al.*, 2010).

Assessment of sap flow density

Sap flow density through the xylem was assessed at breast height by means of the heat balance approach (Granier, 1985). Two sensors per tree were installed on opposing sites and data were logged in 10 min intervals.

Results

System performance

At the reference plot, ambient [CO_2] varied between 353 and 522 µmol mol^{-1} with a mean value (± SD) of 385 ± 22 µmol mol^{-1} (experiment #1). About 20% of observed [CO_2] were > 400 µmol mol^{-1}, and highest concentrations occurred at dawn (Fig A.3A). During the labeling period (Sept.18, 6:00h to Sept. 21, 18:00h), [CO_2] in the sun crown varied between ambient and 770 µmol mol^{-1} and stayed during 60% of the time within a ± 10% range of the target concentration (Fig. A.3A).

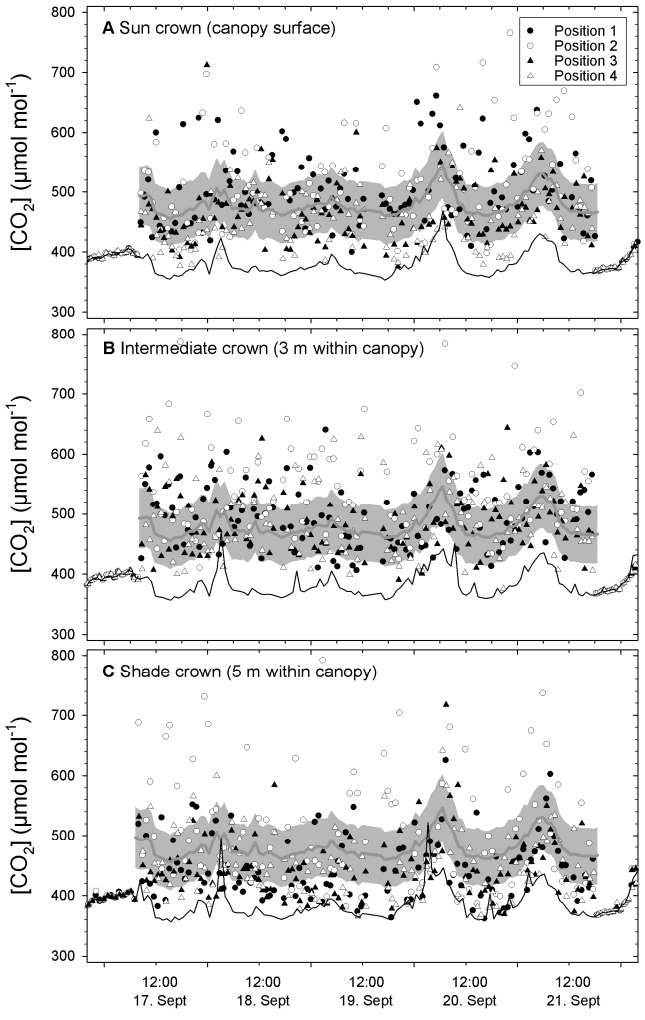


Fig. A. 3: CO_2 concentration in sun (**A**), intermediate (**B**) and shade (**C**) crown of adult beech trees during stable carbon isotope labeling. Different symbols give positions 1 to 4 within the labeling plot (see Fig. A.1A). Thin and thick lines give [CO_2] at the reference plot and target [CO_2] for the isoFACE plot, respectively. Shaded areas represent the ± 10% range of target concentrations. Label was applied continuously from Sept 17, 7:00h to Sept 21, 18:00h.

The same was true in the intermediate crown (sampled at 3 m within the canopy), whereas the [CO_2] in the shade crown (sampled at 5m within the canopy) stayed during 48% of the time within a ± 10% range of the target concentration (Fig. A.3B, C). Highest observed [CO_2] were slightly below 800 µmol mol^{-1} at both intermediate and shade crowns.

In the sun crown, [CO_2] was monitored at higher temporal resolution in 10 s intervals (experiment #2, Fig. A.4). The frequency distribution of instantaneous [CO_2] peaked around 400 µmol mol^{-1} (10% of the observation time). Less than 1% of observations were above 920 µmol mol^{-1}. Averaging the data over periods of 1 and 10 minutes each increased frequencies around 460 µmol mol^{-1} to about 10 and 15%, respectively, (Fig A.4B, C). 10 min means of [CO_2] were restricted to the range of 410 to 670 µmol mol^{-1}.

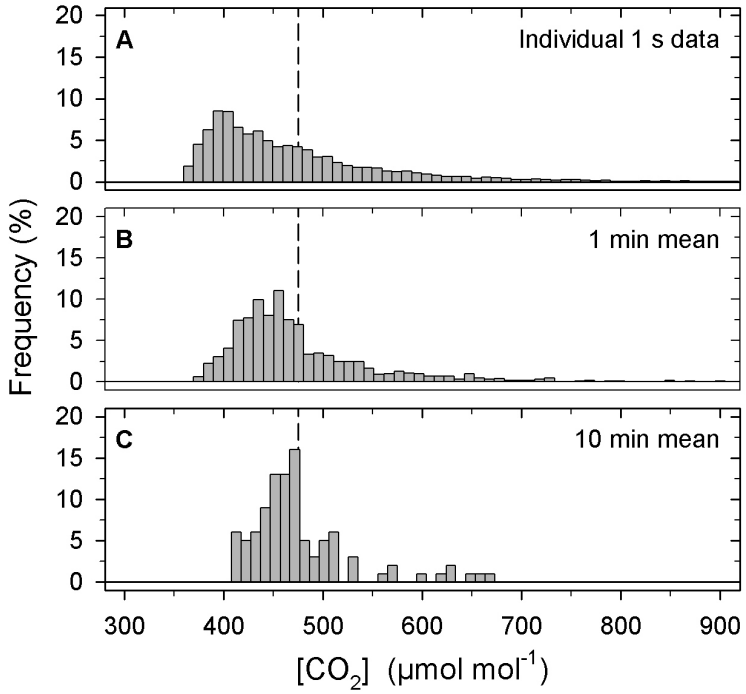


Fig. A. 4: Frequency distribution of [CO_2] assessed in the rough surface of the sun crown of the canopy during experiment #2. Vertical dashed line indicated target [CO_2].

Before and after the labeling, means of [CO_2] over the four sampling positions were similar at the reference and labeling plot. [CO_2] within the canopy was rather constant (381 to 387 µmol mol^{-1}) and somewhat increased 2 m above the forest soil (399 to 415 µmol mol^{-1}) (Table A.1). During labeling, [CO_2] in the sun crown around the photosynthethically most active leaves was increased by 106 µmol mol^{-1} and, hence, close to the target of + 100 µmol mol^{-1}. For intermediate and shade leaves deeper in the canopy, mean [CO_2] was somewhat higher (505 ± 38 µmol mol^{-1}) and lower (461 ± 45 µmol mol^{-1}) than intended, corresponding to increases of 121 µmol mol^{-1} and 75 µmol mol^{-1}, respectively. Comparing the four sampling positions (see numbers 1 to 4 in Fig. A.1B), position 2

consistently reported the highest [CO_2]. Differences were statistically significant in the intermediate and shade crown with mean [CO_2] being 53 and 60 µmol mol^{-1} higher than at the other positions, respectively. Higher [CO_2] at position 2 might have been caused by the vicinity to one of the CO_2 releasing PVC tubes (Fig. A.1B). [CO_2] at 2 m above the forest soil remained unaffected during the labeling of canopy air (Table A.1).

Table A. 1: (**A**) Concentration (µmol mol^{-1}) and (**B**) $\delta^{13}C$ (‰) of atmospheric CO_2 5 m above the canopy and in the sun, intermediate and shade crown of adult beech trees as well as 2 m above the forest soil (under the same trees).

A	**Reference**					**isoFACE**				
	Canopy				Forest soil	Canopy				Forest soil
	Above	Sun	Inter-mediate	Shade	Above	Above	Sun	Inter-mediate	Shade	Above
	(µmol mol^{-1})					(µmol mol^{-1})				
Before	382 (14)	381 (14)	384 (17)	385 (19)	405 (37)	381 (14)	382 (15)	384 (16)	387 (28)	399 (20)
During	380 (20)	381 (21)	384 (23)	386 (27)	390 (27)	388 (21)	488 (45)	505 (38)	461 (45)	392 (26)
After	384 (19)	384 (20)	386 (20)	388 (24)	415 (51)	384 (19)	385 (20)	386 (21)	388 (23)	415 (49)
B	**Reference**					**isoFACE**				
	Canopy				Forest soil	Canopy				Forest soil
	Above	Sun	Inter-mediate	Shade	Above	Above	Sun	Inter-mediate	Shade	Above
	(‰)					(‰)				
Before	-/-	-9.2	-9.1	-9.0	-/-	-8.9 (0.0)	-8.7 (0.2)	-8.9 (0.2)	-9.0 (0.1)	-9.9 (0.7)
During	-9.2 (0.8)	-9.2 (0.6)	-9.5 (0.7)	-9.2 (0.7)	-/-	-11.0 (3.4)	-16.9 (4.2)	-19.3 (5.2)	-13.6 (4.2)	-9.1 (0.5)

Data are presented before, during and after the stable carbon isotope labeling. Data are means (pooled over the five consecutive labeling days of experiment #1 and positions 1 to 4, see Figs. 1B and 3) ± standard deviation (SD); n = 36 to 111 for CO_2 concentration and 4 to 24 for $\delta^{13}C$.

As a result of the CO_2 release into the canopy, mean $\delta^{13}C$ of canopy air was lowered on average by 7.8‰. $\delta^{13}C$ was reduced from about -8.9 to -16.9 (± 4.2), -19.3 (±5.2) and -13.6 (± 4.2) ‰ in the sun, intermediate and shade crown, respectively (Table A.1B). Directly above the forest floor, $\delta^{13}C$ remained unaffected indicating that label CO_2 did not reach the forest soil at about 20 m below the canopy. During daytime $\delta^{13}C$ of the applied CO_2 was estimated by the "Keeling plot" approach as -45.6 ± 0.6‰ (± SE, R^2 = 0.97), which corresponded well with the $\delta^{13}C$ of -46.9‰ of the tank CO_2, although the Keeling plot analysis may be affected by other CO_2 sources such as ecosystem respiration.

Biological proof-of-concept

Before the 19-day labeling period (experiment #3), $\delta^{13}C$ of canopy air at reference and labeling plots were similar, i.e. -8.2 ± 0.2 and -8.4 ± 0.1‰, respectively (Fig. A.5A). The $\delta^{13}C$ of CO_2 efflux sampled from upper and lower stem positions and coarse roots declined with the distance from the crown, i.e. -27.9 ± 0.4 at the upper stem, -28.2 ± 0.1 at breast height and -28.6 ± 0.2‰ in coarse roots. Compared to stem CO_2 efflux at breast height, phloem sugars sampled from the same position had a somewhat reduced $\delta^{13}C$ (-29.1 ± 0.3‰). The reference tree confirmed that environmental conditions over the 19-day labeling period did not induce a change in $\delta^{13}C$ of CO_2 efflux, phloem sugars and soil respired CO_2. Before the start of the labeling the reference tree had displayed somewhat reduced $\delta^{13}C$, in particular, regarding the CO_2 efflux at breast height (i.e. -30.4‰), whereas $\delta^{13}C$ of soil respired CO_2 was slightly higher (-28.1 ± 0.1 compared with -28.8 ± 0.1‰ at the labeling plot). After the 19-day labeling period, $\delta^{13}C$ of canopy air sampled at the reference plot remained unchanged (Fig. A.5B).

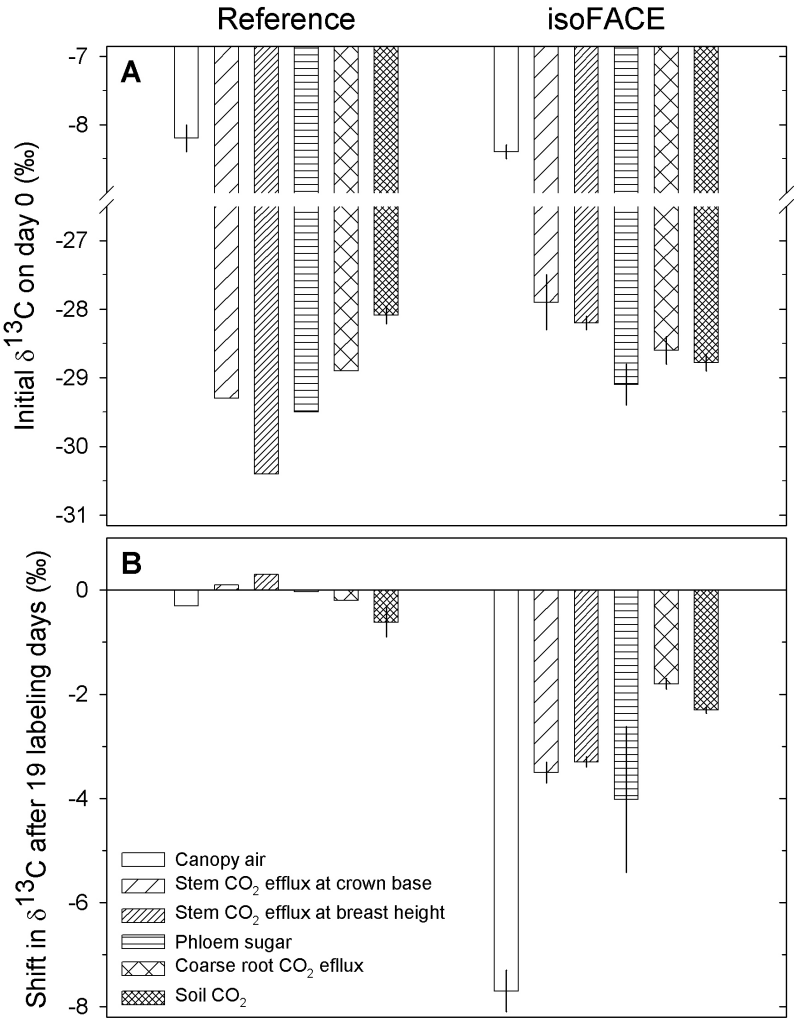


Fig. A. 5: Carbon isotope composition ($\delta^{13}C$) of canopy air, stem CO_2 efflux at crown base and breast height, phloem sugars, coarse root CO_2 efflux and soil respired CO_2 during experiment #1 (**A**) before labeling (day 0) and (**B**) sifts in $\delta^{13}C$ after 19 days of labeling. Data are from three labeled trees. In addition, data from a reference tree is included to confirm environmental conditions do not affect $\delta^{13}C$ during the 19-day labeling period

Carbon labeling of beech canopies resulted in a reduction of $\delta^{13}C$ of the canopy air by 7.8 ± 0.4‰. Subsequently, isotopic signatures of CO_2 efflux and phloem sap sampled from stems were lowered by 3.4 to 4.0‰, whereas $\delta^{13}C$ of CO_2 efflux from roots and soil respired CO_2 was lowered only by 1.8 and 2.3‰, respectively. The shift in $\delta^{13}C$ of soil respired CO_2 and sampled from various organs of the labeled trees give evidence for the uptake of the applied C label. The sap flow density of both reference and labeled trees assessed during a 5-day labeling period (experiment #4) fluctuated with weather conditions, with the highest flow densities occurring during days with high irradiance and VPD (data not shown). Sap flow densities of the reference trees were twice as high in the labeled trees (Fig. A.6B). Most importantly, the ratio of sap flow densities of labeled trees versus those of the reference trees remained unchanged during the 5-day labeling experiment, indicating stomatal conductance of beech trees to be unaffected by the increase in $[CO_2]$ of 100 µmol mol^{-1} (Fig. A.6A).

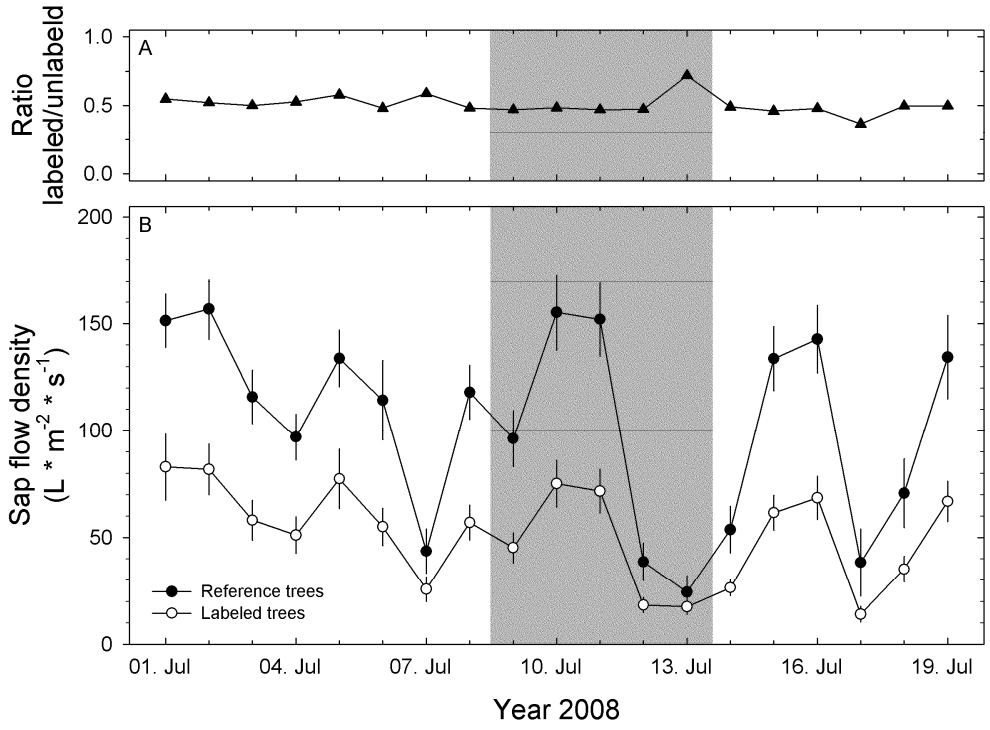


Fig. A. 6: Daily means of sap flow density monitored in stems of labeled (open symbols) and reference (closed symbols) trees. (**A**) Ratio of sap flow density in labeled to reference and (**B**) absolute values of sap flow density. The five-day labeling period is highlighted by the gray area. Data for sap flow density are means of 3 to 4 trees (± SE).

Discussion

In the present work, we detail the set-up and efficacy of a free-air concentration enrichment (FACE) system for the increase of stable carbon isotopes in canopy CO_2, i.e. a stable carbon isotope FACE system, named isoFACE. On average, $\delta^{13}C$ of canopy air was lowered from -8.9 to -16.7‰ by releasing CO_2 with a $\delta^{13}C$ of -46.9‰ into the canopy, while the [CO_2] increase was limited to 100 µmol mol^{-1}. Hence during labeling, mean canopy [CO_2] stayed within the range of the natural fluctuation at the forest site. Subsequent isotopic shifts were traced in phloem sugars, stem and coarse root CO_2 efflux as well as in soil-respired CO_2, documenting the uptake and allocation of the C label by the trees.

The isoFACE system was designed to introduce ^{13}C depleted CO_2 into the canopy of adult forest trees to investigate the fate of C in the atmosphere-tree-soil system. We chose a FACE approach to minimize bias by environmental conditions. Originally, FACE systems have been designed for elevated CO_2 experiments, such as the FACE system of the Brookhaven National Laboratory (Lewin *et al.*, 1992; Hendrey *et al.*, 1993; Hendrey *et al.*, 1999) or subsequently employed infrastructure adopting the original “ring design” (Hendrey & Miglietta, 2006). One-minute averages of [CO_2] were typically 60 to 90% of the time within 10% of the target [CO_2] (Hendrey & Miglietta, 2006). In comparison, the webFACE system releasing CO_2 directly within the canopy from porous tubes stayed within 10% of the target [CO_2] during 47% of time (Pepin & Körner, 2002). Although its primary purpose is on C isotope labeling, the isoFACE system displayed an efficacy of >57% of the time within 10% of the target [CO_2]. Moreover, in less than 1% of observations the instantaneous [CO_2] was > 920 µmol mol^{-1}. Hence, the efficacy of the isoFACE system is comparable (or better) than established FACE systems, and this represents a large improvement in the control of label application compared to earlier free-air stable C isotope exposure systems (cf. Talhelm *et al.*, 2007).

The exposure of CO_2 directly within the canopy through vertically hanging tubes has several advantages, including a reduction in [CO_2] gradients within the canopy and improved exposure control during days with low wind speed (cf. Karnosky *et al.*, 2007; Pepin & Körner, 2002; Werner & Fabian, 2002). The applied design is in need of a supportive structure for tube installation. Low-weight construction material such as surf-boat masts can meet that need, allowing for mounting the supportive structure directly to the straight stems of coniferous trees, as successfully tested already during more recent experiments (not shown). One putative limitation may be the required increase in [CO_2] during label application. In the present study, we limited this increase on average to 100 µmol

mol^{-1}, and most of the time (>75%) canopy [CO_2] stayed within the natural variation observed at the reference plot (cf. Berry *et al*., 1997; Mortazavi & Chanton, 2002). To further limit a putative [CO_2] effect, less label could be applied. Limitation of the increase of [CO_2] to 50 µmol mol^{-1} would still change $\delta^{13}C$ of canopy air by about 4‰. Nevertheless, a putative effect of the increase in [CO_2], e.g. on the C transfer through the trees-soil system, may be assessed. To this end, a parallel short-term pulse of highly enriched $^{13}CO_2$ could be applied to both the control trees and those which have been labeled by the isoFACE system. The present increase in [CO_2] by about 100 µmol mol^{-1} did not affect sap flow densities of labeled beech trees, suggesting unchanged stomatal aperture at the leaf level. This is confirmed by findings of Keel *et al*. (2007) that adult beech trees display low response in stomatal conductance under elevated [CO_2]. Similarly, low responsiveness to changes in atmospheric [CO_2] was observed in conifers (Gunderson *et al.*, 1993; Norby *et al.*, 1999). At the same time the ratios of leaf internal to leaf external [CO_2] of juvenile and adult beech (Grams *et al.*, 1999; Grams, 2007, Markus Löw, Center for Plants and Environment, University of Western Sydney, Australia, pers. communication) are only slightly reduced (< 0.02) by an increase in canopy [CO_2] of 100 µmol mol^{-1}. Hence, changes in discrimination of $^{13}CO_2$ by photosynthesis are assumed to be below 0.4‰ (Farquhar *et al.*, 1989).

Compared to recent short-term approaches which use enclosures for highly enriched ^{13}C-labeling of canopy air (Högberg *et al*., 2008; Plain *et al*., 2009), the isoFACE system was designed for longer labeling experiments. Quantification of C fluxes, time constants and identification of metabolites are facilitated in homogeneous, long-term labeling approaches and may not be achieved during short-term pulse labeling (deVisser *et al.*, 1997; Thornton *et al.*, 2004). Instead, long-term, steady-state labeling is advocated as the method of choice for quantitative analyses of resource partitioning (deVisser *et al*., 1997).

In a long-term labeling approach, a prerequisite for tracing labeled C in soil-respired CO_2 is that CO_2 applied at the canopy level does not directly reach the forest floor and subsequently diffuse into the soil. We can exclude such a downward movement of the applied CO_2 because [CO_2] and $\delta^{13}C$ above the forest floor remained unchanged during the labeling (Table A.1, Andersen *et al*., 2010). This conclusion is in agreement with Steinmann *et al.* (2004), who neither observed direct diffusion of canopy CO_2 towards the forest soil during the operation of the webFACE system. The unchanged isotopic signature of the understory air highlights the advantage of restricting label application to the

canopy level in comparison to approaches where the soil surface is exposed to the applied C label (Högberg *et al*., 2008; Ostle *et al*., 2007; Subke *et al*., 2009).

The isoFACE system displayed distinctly improved homogeneity of label application compared to an earlier free-air C labeling system for small trees (Talhelm *et al*., 2007). Nevertheless, $\delta^{13}C$ of canopy air deviated by 2 to 3‰ in lower canopy layers compared to the sun crown. At the investigated research site, previous studies demonstrated that C fixation of beech canopies is dominated by the contribution of the sun crown, whereas intermediate and shaded parts of the crown were of minor importance (Reiter *et al.*, 2005; Grams, 2007; Kitao *et al.*, 2009). We therefore assume that C fixed and exported from the canopy during the labeling corresponds to the label strength in the sun crown, i.e. reduction of 7.8‰ (from -8.9 to -16.7‰). In comparison, $\delta^{13}C$ of phloem sugars and stem CO_2 efflux at both stem heights were lowered by only 3.4 to 4.0‰, indicating that only half of the sampled C originated from C fixed during the 19 labeling days. This highlights the contribution of non-labeled C in phloem sugars that may originate from remobilized C or from "old" C atoms in the C skeletons of recently synthesized sucrose as a consequence of slow turned-over of precursor molecules (Tcherkez *et al.*, 2003; Gessler *et al.*, 2009b). CO_2 sampled from stem efflux may not exclusively originate from local respiration behind the sampling chambers since CO_2 dissolved in and transported with the xylem sap is known to contribute to stem CO_2 efflux (Teskey *et al.*, 2008). However, $\delta^{13}C$ in phloem sugars and stem CO_2 efflux were shifted to a similar extent, indicating that phloem sugars represent the dominating C source for CO_2 diffusing out of the stem tissue. This confirms recent findings that transport of CO_2 in xylem sap has negligible influence on $\delta^{13}C$ of CO_2 efflux (Kodama *et al.*, 2008; Ubierna *et al.*, 2009; Kuptz *et al.*, 2011b). In the case of coarse roots, the drop in $\delta^{13}C$ in response to label application was smaller (< 2‰), reflecting reduced dependence of root respiration on respiratory substrates (Dieuaide-Noubhani *et al.*, 1995; Wingate, 2008; Bathellier *et al.*, 2009). In a similar way, soil-respired CO_2 displayed reduction in $\delta^{13}C$ of about 2.5 ‰, which also reflects large contributions by roots of neighboring, non-labeled trees and heterotrophic soil respiration (Högberg *et al.*, 2001; Andersen *et al.*, 2005; Andersen *et al.*, 2010).

In conclusion, the design of the isoFACE system employing micro-porous tubes hanging vertically at approx. 1 m distance through the canopy allows for long-term C isotope labeling, including periods of low wind velocity. High efficacy was demonstrated with stable carbon isotopes but the isoFACE system could also be employed to apply ^{14}C-labeled CO_2. In the present paper, a reduction of $\delta^{13}C$ of canopy air by 7.8‰ was achieved, while increase of canopy [CO_2] was limited to 100 µmol

mol^{-1}. [CO_2] stayed within 10% of the target [CO_2] during >57% of the time. Successful label application and allocation by the tree was demonstrated by monitoring $\delta^{13}C$ of canopy air and sampling of phloem sugars, stem and coarse root CO_2 efflux and soil respired CO_2. Even after 19 days, only about half of the C in CO_2 efflux of stems was labeled, indicating the rather slow turn-over of a presumably large pool of respiratory substrates involved in woody tissue respiration (Wingate *et al.*, 2010). In coarse roots, this respiratory turn-over was found to be even slower and only about 30% of C was labeled at the end of the labeling period.

Steady-state C labeling through the isoFACE infrastructure will facilitate quantitative assessment of C transport rates, turn-over and pools sizes of involved metabolites in the trees-soil system (Kuptz *et al.*, 2011a). This will be of particular interest in studies on the coupling between CO_2 fixation and soil or ecosystem respiration (Kuzyakov & Gavrichkova, 2010). In addition, long-term labeling will allow for sampling of plant material to analyze labeling kinetics of involved metabolites and respiratory substrates. By this, the C flux through forest trees and ecosystems can be traced in great detail from photosynthetic CO_2 fixation, over involved metabolites to the respiratory release or structural incorporation.

Appendix B

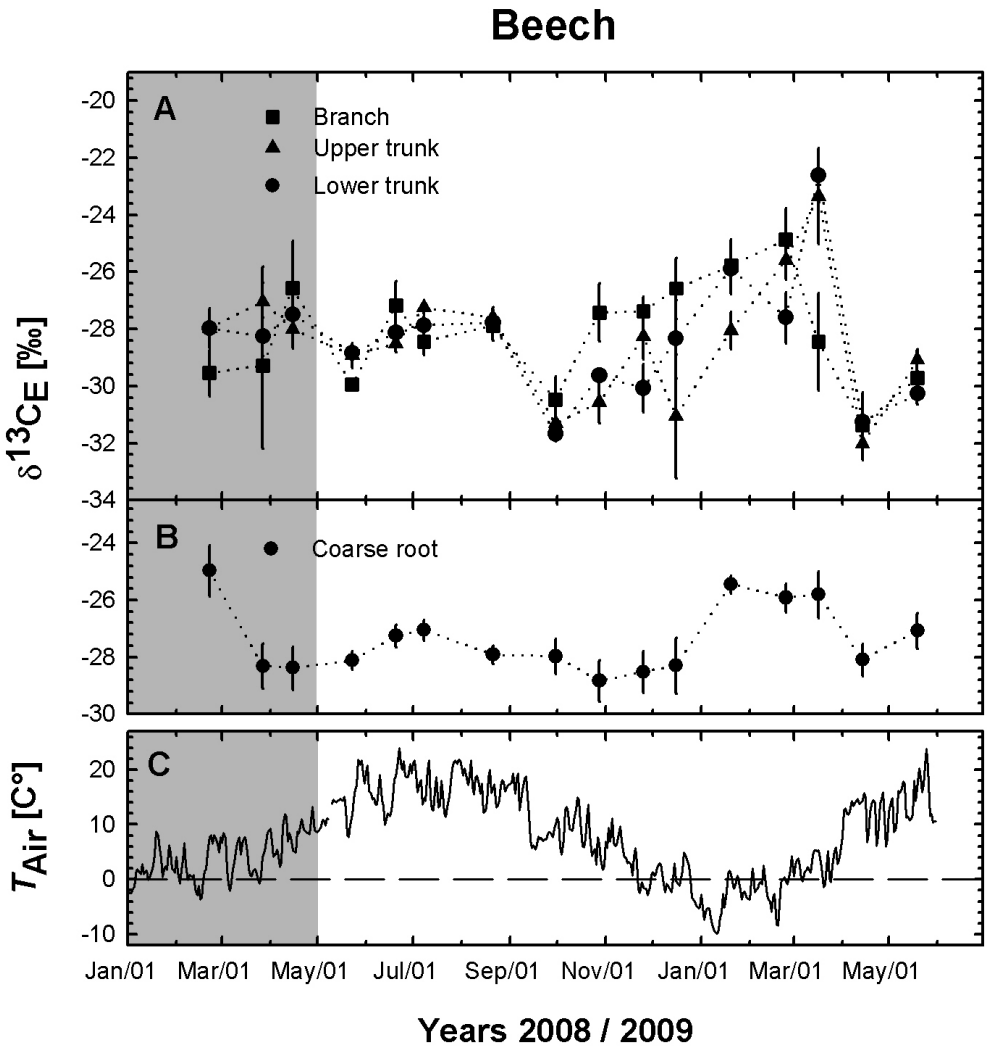


Fig. B. 1: Annual changes in $\delta^{13}C$ of CO_2 efflux ($\delta^{13}C_E$) of non-leafy branches, trunks and coarse roots of beech (closed symbols, **A + B**) including measurements from February till April 2008 and mean daily air temperature (T_{Air}, **C**). Data in A + B give daily means ± SE. From February till April 2008 branch and trunk chambers were not darkened (symbolized by gray area). During winter dormancy 2008, $\delta^{13}C_E$ values of above ground tree components did not increase as during winter dormancy 2009 and resembled values during the main vegetation period. Similar, Wingate *et al*. (2010) did not observe differences between winter and summer $\delta^{13}C_E$ of *Pinus sylvestris* stems. In both cases, winter temperature was rather mild and daily mean T_{Air} stayed above 0 ℃ for most of the time (> 80%). Thus, TCA cycle activity may have been higher compared to winter dormancy 2009, dampening the PEPc induced accumulation of malate, which was discussed in chapter 2.

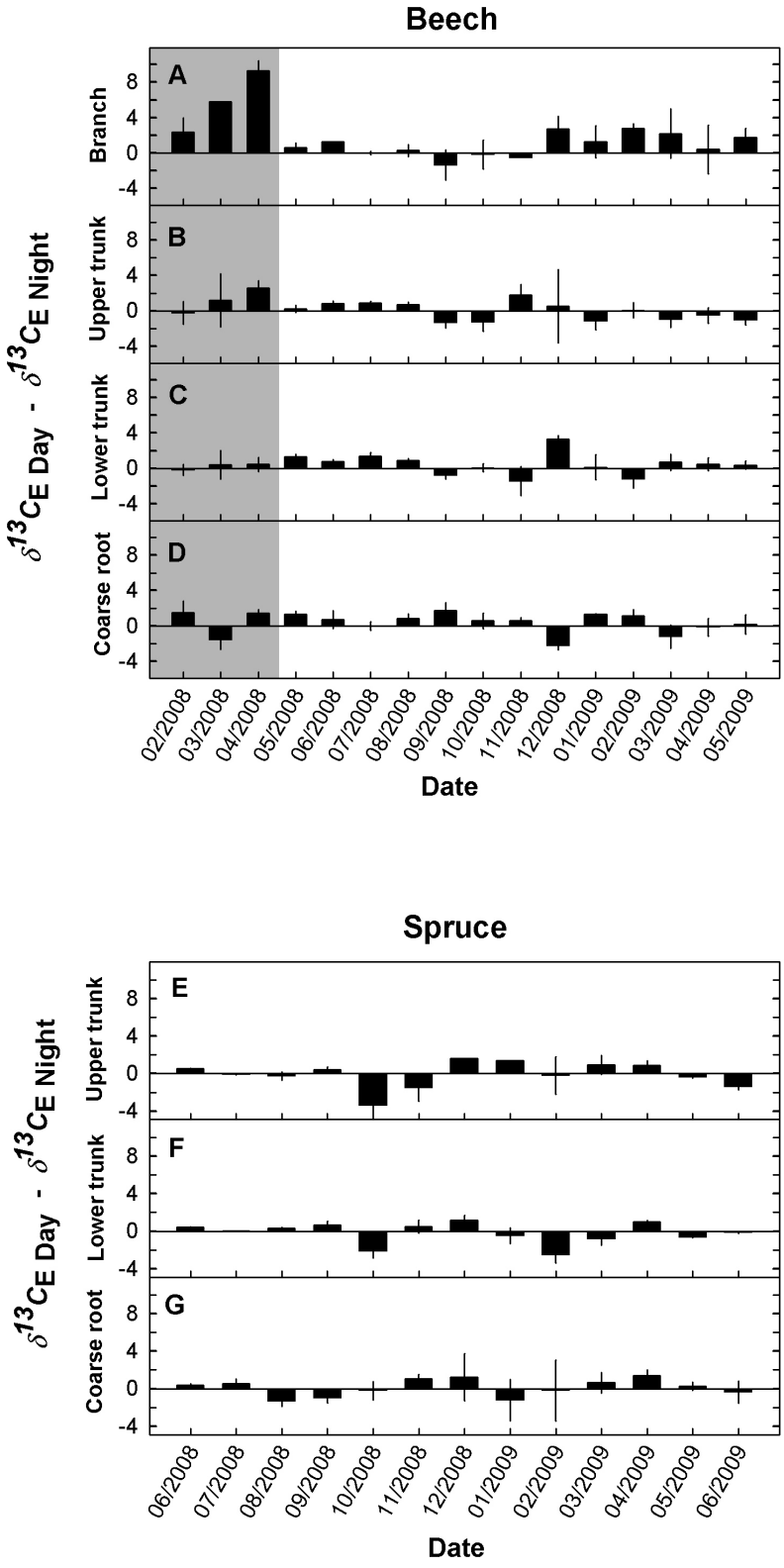


Fig. B. 2: Difference in $\delta^{13}C_E$ during the day and $\delta^{13}C_E$ during the night (mean ± SE) of beech (**A – D**) and spruce (**E – G**) branch, trunk and coarse root CO_2 efflux. From February till April 2008 branch and trunk chambers of beech were not darkened (symbolized by gray area). Diurnal difference between day and night $\delta^{13}C_E$ increased in uncovered branches from February till April 2008 (9.3 ± 1.1‰), as photosynthetic discrimination might have occurred in chambers during CO_2 fixation by photosynthetic bark at rising temperatures (Cernusak *et al.*, 2001).

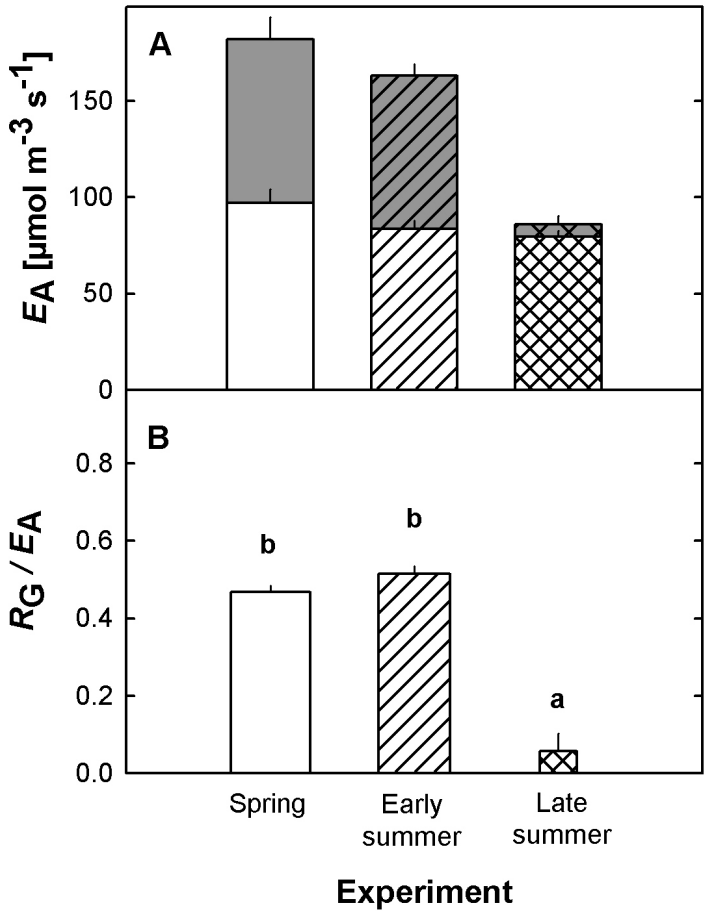


Fig. B. 3: (**A**) CO_2 efflux rate (E_A) of branches of beech during three labeling experiments. Estimated maintenance respiration (R_M) is represented by white bars, E_A by the sum of white and gray bars. Data shown are means (± SE) of all labeled trees during the experiments. (**B**) Quotient of growth respiration (R_G) to E_A for beech branches during labeling experiments. Bar width in B gives relative CO_2 efflux rate of branches between experiments. Lower case letters symbolize significant differences between experiments with $p \leq 0.001$. E_A was maximal during spring and decreased until late summer (182 ± 11 and 86 ± 4 µmol m^{-3} s^{-1}, respectively). Growth respiration was high during spring and early summer and declined during late summer. Likewise, R_G/E_A was significantly decreased in late summer.

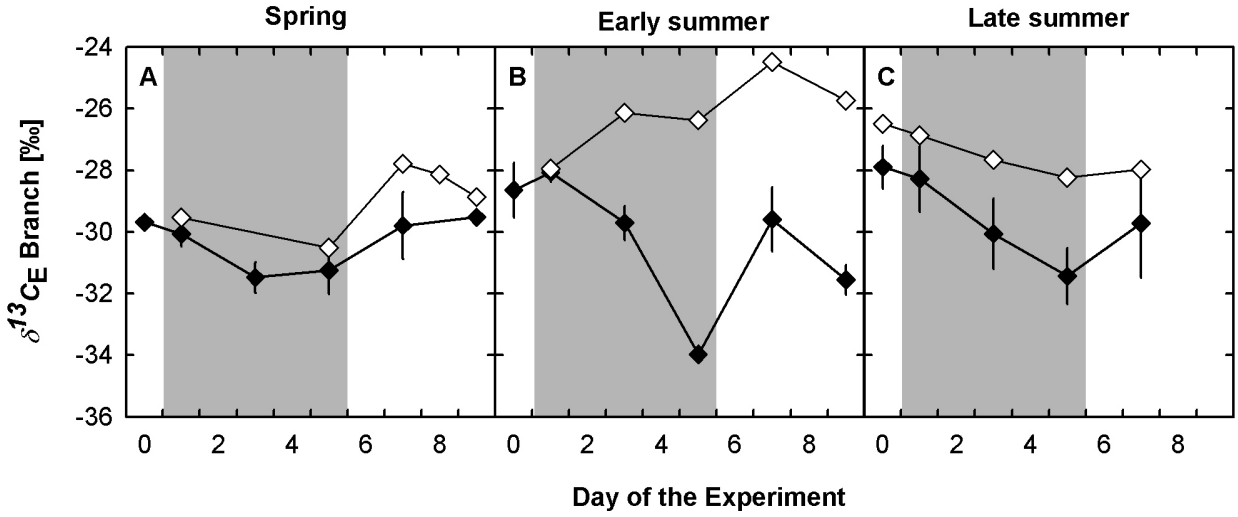


Fig. B. 4: Stable C isotopic composition of branch CO_2 efflux ($\delta^{13}C_E$) during labeling experiments (**A** = spring, **B** = early summer, **C** = late summer) of beech. Data are shown as daily means ± SE. Closed symbols represent labeled trees (n = 4), open symbols the control tree (n = 1). Gray area represents time of label application. Data were collected once per day every second day with the closed-respiration system as described in chapter 2, 3 and appendix A. Label induced shift was maximal in early summer (-6.9 ± 0.0 ‰) and low during spring (-1.8 ± 1.0 ‰) and late summer (-1.8 ± 0.9 ‰).

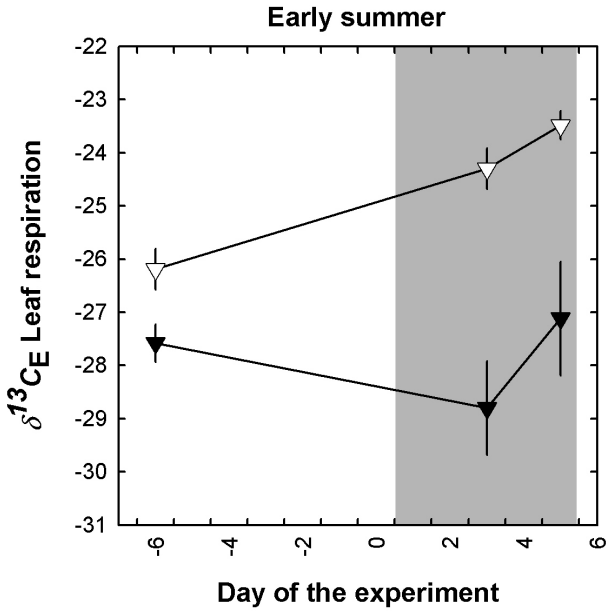


Fig. B. 5: Stable C isotopic composition of leaf CO_2 efflux ($\delta^{13}C_E$) during labeling experiment the early summer of beech. Data are shown as daily means ± SE. Closed symbols represent labeled trees (n = 2 leaves x 4 trees), open symbols control tress (n = 2 leaves x 3 trees). Gray area represents time of label application. Leaves were sampled once per day and $\delta^{13}C_E$ was assessed as described in Werner et al. (2007). Immediately after sampling, leaves were enclosed into 12 ml glass vials (Exetainer®, Labco Limited, High Wycombe, UK). Vials were flushed with CO_2 free air and leaves were darkened for at least 30min. Respiratory CO_2 was transferred into an empty vial using a syringe and measured for $\delta^{13}C_E$ by IRMS. Label application resulted in a shift in leaf $\delta^{13}C_E$ of maximal -3.1 ± 0.9 ‰ at day three of the experiment.

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Candidate's individual contribution

Chapter 2

Kuptz D, Matyssek R, Grams TEE (2011) "Seasonal dynamics in the stable carbon isotope composition ($\delta^{13}C$) from non-leafy branch, trunk and coarse root CO_2 efflux of adult deciduous (*Fagus sylvatica*) and evergreen (*Picea abies*) trees", *Plant, Cell & Environment* resubmitted after review (previous decision was "accepted minor")

The candidate developed the research question and the story of this paper with help of his coauthors. He installed most parts of the flow-through gas-exchange chamber system and conducted the measurements. He analyzed, interpreted and discussed the results. He composed the graphs, wrote the first draft of this paper, worked in the correction suggestions by his coauthors and submitted the publication to *Plant, Cell & Environment*. After review (first decision: "reject encouraged", second decision: "accepted minor"), he worked in the comments of the referees and resubmitted the publication to the journal.

Chapter 3

Kuptz D, Fleischmann F, Matyssek R, Grams TEE (2011) "Seasonal patterns of carbon allocation to respiratory pools in 60–year-old deciduous (*Fagus sylvatica*) and evergreen (*Picea abies*) trees assessed via whole-tree stable carbon isotope labeling", *New Phytologist*, submitted for publication

The candidate developed the research question and story of this paper with help of his coauthors. He installed most parts of the flow-through gas-exchange chamber system, the isoFACE infrastructure on spruce and also partly on beech. He conducted the measurements and analyzed, interpreted and discussed the results. Measurements and interpretation on phloem sugars were done with help by Frank Fleischmann. The candidate composed the graphs, wrote the first draft of this paper, worked in correction suggestions by his coauthors and submitted it to *New Phytologist*.

Appendix A

Grams TEE, Werner H, **Kuptz D**, Ritter W, Fleischmann F, Andersen CP, Matyssek R (2011) "A free-air system for long-term stable carbon isotope labeling of adult forest trees" *Trees – Structure and Function*, DOI: 10.1007/s00468-010-0497-7

The candidate installed parts of the flow-through gas-exchange chamber setup and the isoFACE infrastructure. He contributed to the measurements on label survey in canopies, label survey on the forest floor and sap flow rate. He helped to interpret and discuss the results and corrected parts of the first draft by Thorsten Grams.

Curriculum vitae

Personal data

Name	Daniel Kuptz
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Education

August 2007 – October 2010	Doctorate at the Chair of Ecophysiology of Plants, Technische Universität München, Freising, Germany: Titel of PhD thesis: „Seasonal dynamics behind the CO_2 efflux of adult European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*) trees – uncovered by stable C isotopes”
October 2002 - July 2007	Study of Forestry Science at the Technische Universität München, Freising, Germany Titel of Diploma thesis at the Chair of Silviculture: „Zuwachs und Photosynthese von Anreicherungspflanzungen in einem tropischen Bergregenwald Südequadors“ with experimental part at the „Reserva Biologica San Francisco“, Loja, Ecuador Degree: Forestry science engineer (Dipl. Univ.) (July 2007)
2001 - 2002	Community service at nursing home “Ernst-Faber Haus”, Coburg, Germany
1993 - 2001	Gymnasium Albertinum, Coburg, Germany Degree: Abitur
1992 - 1993	Ernst-Moritz-Arndt Gymnasium, Remscheid, Germany
1988 - 1992	Primary school „Grundschule Siepen“, Remscheid, Germany

Personal publications

Kuptz et al. 2010	**Kuptz D**., Grams TEE., Günter S. (2010) „Light acclimation of four native tree species in felling gaps within a tropical mountain rainforest“, *Trees – Structure and Function* 24, 117 - 127
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Grams et al. 2011	Grams TEE., Werner H., **Kuptz D**., Ritter W., Andersen CP., Matyssek R. (2010) – „ A free-air system for long-term stable carbon isotope labeling of adult forest trees” *Trees – Structure and Function*, DOI: 10.1007/s00468-010-0497-7
Kuptz et al. 2011	**Kuptz D**., Fleischmann F., Matyssek R., Grams TEE. (2010) „ Seasonal patterns of carbon allocation to respiratory pools in 60–year-old deciduous (*Fagus sylvatica*) and evergreen (*Picea abeis*) trees assessed via whole-tree stable carbon isotope labeling”, *New Phytologist*, submitted
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Conferences

July 2009	**Kuptz D**., Matyssek R., Grams TEE. “Stable C isotope composition of CO_2 efflux of different tree components reveals annual and diurnal dynamics of carbon use in adult European beech and Norway spruce trees” at “The use of stable isotopes in tree physiology and forest ecology”, Nancy, France, presentation
September 2009	**Kuptz D**., Matyssek R., Grams TEE. “Stable C isotope composition of CO_2 efflux of different tree components reveals annual and diurnal dynamics of carbon use in adult European beech and Norway spruce trees “ at “GFÖ 2009 – Dimensions of ecology from global change to molecular ecology”, Bayreuth, Germany, presentation
March 2010	**Kuptz D**., Matyssek R., Grams TEE “Seasonal patterns in allocation to respiratory C pools in 60–year-old trees” at “4th International Conference on mechanisms of growth, competition and stress defense in plants”, Freising, Germany, poster
March 2010	**Kuptz D**., Matyssek R., Grams TEE “Seasonal patterns in allocation to respiratory C pools in 60–year-old trees” at “Stable Isotopes and Biogeochemical Cycles in Terrestrial Ecosystems”, Ascona, Switzerland, poster

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Freising, den

Daniel Kuptz